

# **TURBIDITY STANDARDS**

Technical Information Series — Booklet No. 12

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In memory of  
**Clifford C. Hach**  
(1919-1990)

inventor, mentor, leader and, foremost,  
dedicated chemist

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## Introduction

### Importance of Standards

All scientific work depends on precise measurement to assure meaningful results. British mathematician and physicist Lord Kelvin expressed the necessity of accurate measurement over 100 years ago: “If you can measure that of which you speak, and you can express it in number, you know something of your subject; but if you cannot measure it, your knowledge is meager and unsatisfactory.”

Standards are the foundation of scientific measurement, the fundamental units in which we describe our work. They provide the terms we need to express conditions, procedures, and results, serve as a reference against which other measurements are compared, and establish a basis for compatibility in communication of scientific work throughout the world.

### Definition of Primary Standard

Physical measurements such as length, weight, mass, and volume are based on *primary* standards. Primary standards, once defined, require no further reference. The primary standard of length, the meter unit, is defined as being equal to 1,650,763.73 wavelengths of the orange-red light emitted by Krypton-86, and the primary standard of volume, the liter, is *defined* as the space occupied by 1 dm<sup>3</sup>=10<sup>-3</sup> m<sup>3</sup>.

Some primary standards are by agreement. For instance, in an alternative definition above of the standard for volume, another unit — the kilogram — is used. A specific bar of platinum-iridium metal is used as the world’s accepted definition of a kilogram, the unit of mass. In chemistry, primary standards are often defined by repeatable experiment. For instance, a primary standard can be a compound that can be prepared repeatably with acceptable purity and stability. As the term “primary” implies, its preparation results in establishment of the reference, the basis by which all other measurements are taken. Once the primary standard is defined, no other reference is necessary.

### Turbidity Standards

The subject of standards in turbidimetric measurement is complicated partly by the variety of types of standards in common use and acceptable for reporting purposes by organizations such as the USEPA<sup>1</sup> and *Standard Methods*, and partly by the terminology or definition applied to them.

In the 19th edition of *Standard Methods for the Examination of Water and Wastewater*, clarification was made in defining primary versus secondary standards. *Standard Methods* defines a primary standard as one that is prepared by the user from traceable raw materials, using precise methodologies and under controlled environmental conditions. In turbidity, the only standard that can be defined as primary is formazin that has been prepared by the user on the bench.

*Standard Methods* now defines secondary standards as those standards a manufacturer (or an independent testing organization) has certified to give instrument calibration results equivalent (within certain limits) to results obtained when an instrument is calibrated with user-prepared formazin standards (primary standards). Various standards that are suitable for calibration are available, including commercial stock suspensions of 4000 NTU formazin, stabilized formazin suspensions (StablCal™ Stabilized Formazin Standards, also referred to as StablCal Standards, StablCal Solutions, or StablCal), and commercial suspensions of microspheres of styrene divinylbenzene copolymer.

Calibration verification items supplied by instrument manufacturers, such as sealed sample cells filled with a latex suspension or with metal oxide particles in polymer gel, are used to check a calibration and are not to be used in performing instrument calibrations.

If there is a discrepancy on accuracy of a standard or an instrument, the primary standards (i. e., user-prepared formazin) are to be used to govern the validity of the issue. In turbidity, formazin is the only recognized true primary standard and all other standards are traced back to formazin. Further, instrument algorithms and specifications for turbidimeters should be designed around this primary standard.

Currently, the USEPA designates user-prepared formazin, commercial stock formazin suspensions, and commercial styrene divinylbenzene suspensions (sometimes referred to as “alternative standards”) as primary standards and usable for reporting purposes. The term secondary is used for those “standards” that are used only to check or verify calibrations. Under this USEPA definition, primary does not have anything to do with traceability, only to acceptability for USEPA reporting purposes.

Primary standards are also used for measuring and determining the value of all other standards. Under the USEPA definition, secondary standards, once their values are determined versus primary formazin, are used to verify the calibration of a turbidimeter. However, these standards are not to be used for calibrating instruments. Examples of these standards include the metal oxide gels, latex, and any non-aqueous standards that are defined to monitor calibrations on a day-to-day basis. This usage depends on the design of the standard. On the other hand, formazin, StablCal standards and Amco AEP-1 Alternative Standards are designed to calibrate the instruments.

In the discussion that follows, we will examine the meaning and importance of primary and secondary standards of turbidity, various commercial forms and their proper use, and alternative standards. We will consider the advantages and disadvantages associated with each.

<sup>1</sup>United States Environmental Protection Agency

## Development of the Primary Turbidity Standard

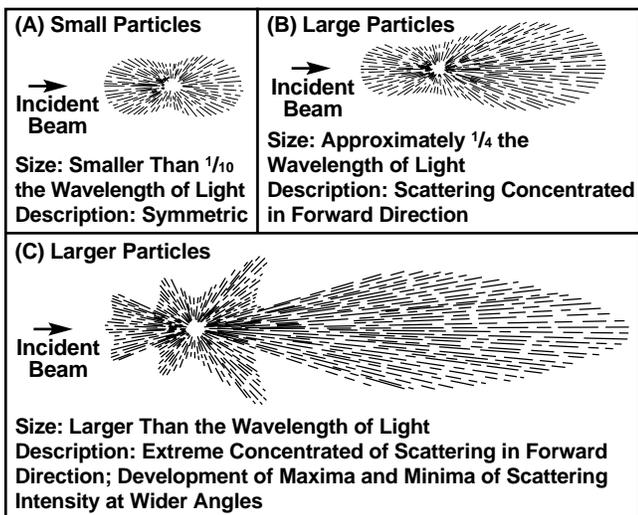
### Definition of Turbidity

By definition, turbidity is an “optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample.”<sup>2</sup> Scattering and absorption are caused when undissolved particles such as silt, clay, algae, organic matter, and microorganism suspended in a sample interfere with light passing through. Their effect is to impart a haze or cloudiness to the sample. Simply defined, turbidity is the opposite of clarity.

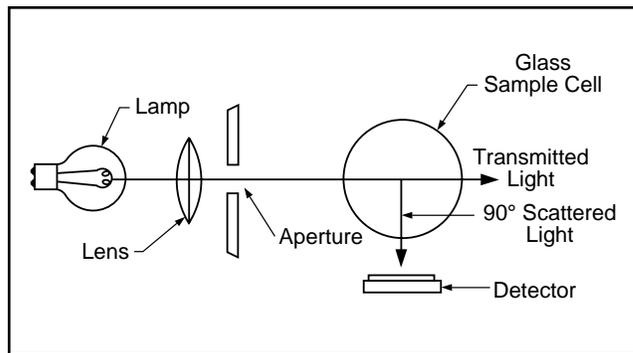
It is possible to quantify turbidity by measuring the amount of light scattered away from the direction of incident light, or the amount of light absorbed from the incident beam. In order for either of these measurements to be meaningful, however, it is necessary to have a frame of reference, or standard, against which the numbers can be compared.

### Turbidity Units of Measure

Establishing a standard for turbidity measurement and defining its units is not as simple a process as it is for many chemical measurements. Turbidity is a qualitative property, usually caused by a wide variety of substances. Light-scattering properties of a given particle depend on its size, shape, and refractive index (see Figure 1). Extremely small particles scatter short wavelength light differently than long wavelength light. As particle size increases, the effect is the opposite. The complex nature of the scattering effect and the number of variables involved make absolute comparisons difficult.



**Figure 1:** Scattering is a function of light wavelength and particle size. Assuming a wavelength of approximately 600 nm, the figure shows angular patterns of scattered light from particles of three relative sizes: A) < 60 nm, B) ≈ 150 nm, and C) ≈ 6000 nm. From Brumberger et al, “Light Scattering”, *Science and Technology*, November, 1968, page 38.



**Figure 2:** A nephelometer quantifies turbidity by measuring light scattered by suspended particulate material. This configuration detects light scattered at 90° from the incident beam.

Early efforts to quantify turbidity were done by Whipple and Jackson in 1900. Their work involved the formulation of a standard suspension of 1000 parts per million of diatomaceous earth in distilled water. Although the suspension could not be formulated repeatedly using materials from different sources, this was the first relative scale of comparison for turbidity measurements. Compared to the standard suspension, a sample could be described as having the same turbidity as X parts per million suspended silica in distilled water. Units of “parts per million, silica turbidity” were used, and are still found in references today. Because this was an equivalent scale and did not relate to a specific quantity of matter in a sample, the unit of measure was later redefined as a Turbidity Unit (TU), the basic unit of measure accepted today.

Turbidity Units are usually stated with a qualifier that specifies the method of measurement. A nephelometer measures light scattered by a sample in the direction that is 90 degrees from the incident light path (see Figure 2); turbidity measured in this way is stated in Nephelometric Turbidity Units (NTU). Visual extinction methods of measurement such as the Jackson Candle Turbidimeter method measure attenuation of light in the direction of the incident beam; these methods report in Jackson Turbidity Units (JTU). Note that although both units have the same basis, results derived by the two measurement techniques can differ substantially for the same sample.

### Formazin as a Primary Turbidity Standard

Defining a unit of measure and a relative calibration scale was a key development in turbidity measurement. The major remaining difficulty was that the standard suspension could not be formulated repeatably when using natural materials from different sources. Many organic and inorganic substances were proposed for use in preparing a primary turbidity standard. Formazin, a polymer suspension, was proposed for the role as early as 1926, but little research resulted. It wasn’t until the early 1960s that Johns-Manville Corporation representatives contacted Hach Company and also suggested formazin.

<sup>2</sup>Standard Methods for the Examination of Water and Wastewater, 19th edition, 1995, page 2-8.

Pursuing their suggestion, Hach chemists found that formazin, prepared by combining solutions of hydrazine sulfate and hexamethylenetetramine, could indeed meet all the requirements of a primary standard. Under the correct conditions, formazin could be prepared as a pure compound, its preparation was reproducible to within  $\pm 1\%$ , and it was relatively stable and easy to use.

Additional work led Hach researchers to define a particular formazin suspension, equal volumes of a 1 gram/100 mL solution of hydrazine sulfate and a 10 gram/100 mL solution of hexamethylenetetramine, as a 4000 NTU standard. Later, American Public Health Association (APHA) and American Water Works Association (AWWA) committees accepted this system. Formazin was specified as the primary turbidity standard beginning with *Standard Methods*, 13th edition, 1971. The United States Environmental Protection Agency (USEPA) has also specified formazin as the primary turbidity standard.

Formazin has several desirable characteristics that make it an excellent analytical standard. First, it can be reproducibly prepared from assayed raw materials. Second, the physical characteristics make it a desirable light-scatter calibration standard. The formazin polymer consists of several different length chains which fold into random configurations. These result in a wide array of particle shapes and sizes ranging from  $<0.1$  to  $>10$  microns. Studies on the particle distribution indicate irregular distributions among different lots of standards, but the overall statistical nephelometric scatter is very reproducible. This wide array of particle sizes and shapes analytically fits the wide possibility of particle sizes that are found in real world samples. Due to the statistical reproducibility of the nephelometric scatter of white light by the formazin polymer, instruments with the traditional tungsten filament white light optical designs can be calibrated with a high degree of accuracy and reproducibility. The randomness of particle shapes and sizes within formazin standards yield statistically reproducible scatter on all makes and models of turbidimeters.

Since real-world samples have a wide distribution of particle sizes and shapes, the ideal turbidity standard should encompass this variability. Formazin, with its broad particle distribution, is the one turbidity standard that will encompass the particle shapes and sizes of most aqueous samples. Other standards that have very narrow particle distributions and the same particle shapes may very well be outside the constraints of many real-world samples. This feature can lead to unsuspected error in turbidity measurement. Attempts have been made to replicate the size and distribution of formazin standards and have had only limited success. Further, the production of such standards is complex, time consuming and costly.

Formazin is not without its limitations. The starting raw materials used in the synthesis of the formazin polymer, hydrazine sulfate and hexamethylenetetramine (hexamine), are currently listed as a suspected carcinogen and an experimental mutagen respectively. However, in the final synthesized stock formazin polymer solution (4000 NTU) and in any dilutions of this stock standard, these two starting raw materials are in very low concentrations. Thus, the exposure level of these starting raw materials is at exponentially lower levels in the final calibration standards containing the synthesized formazin polymer. When the quantities of these starting raw materials in the finished stock standard and subsequent dilutions are compared to the concentration of the starting raw materials present before the synthesis of the polymer, the quantity of hydrazine sulfate is several orders of magnitude lower in the finished standards. An independent analysis for hydrazine sulfate in formazin calibration standards concluded that there is between  $10^{-2}$  and  $10^{-3}$  times less hydrazine sulfate present than the amount present at the beginning synthesis step of the formazin polymer. There is no strong supporting evidence on the hazardousness claims on the finished formazin calibration standards.

A second limitation of formazin is its stability. Formazin is only stable at high concentrations. In-house stability studies indicate that formazin standards above 400 NTU are stable for greater than one year, standards between 20 and 400 NTU are stable for approximately one month, standards between 2 and 20 NTU are stable for approximately 12 to 24 hours; and, standards below 2 NTU are stable for 1 hour or less. Hach does not recommend user preparation of formazin standards below 2 NTU.

When an analyst would like to calibrate or verify an instrument calibration in the measurement range of interest, the preparation of a low-turbidity formazin standard is extremely difficult. Particle contamination is usually imminent and extreme preventive precautions must be taken through every step of this preparation process. Due to the care that must be taken, preparation of low-turbidity formazin standards is a very tedious, difficult and timely process.

## Using Formazin Standards

### Preparation of Formazin Stock Suspension

Formazin is an aqueous suspension of an insoluble polymer formed by the condensation reaction between hydrazine sulfate and hexamethylenetetramine (see Figure 3). As stated previously, a 4000 NTU suspension is prepared by combining equal volumes of a 1.000 g/100 mL solution of hydrazine sulfate and a 10.0 g/100 mL solution of hexamethylenetetramine. After standing at  $25 \pm 1^\circ\text{C}$  for 24 hours, the solution develops a white particulate suspension. *Appendix II* describes the complete procedure for preparing dilute formazin standards.

The 4000 NTU formazin suspension can be reproduced with a high degree of accuracy, and is very stable when stored properly (see below). However, formazin preparation is affected by a number of variables, including purity of materials, temperature and exposure to light. These factors must be accounted for to assure reproducibility and accuracy. For convenience, analysts may wish to work with commercially-prepared 4000 NTU formazin standards. Prepared under the proper conditions, these standards ensure repeatability (1% lot-to-lot) for the greatest accuracy and consistency in measurement and reporting.

### Preparation of Formazin Calibration Standards

Calibration or “working” standards may be prepared by diluting a 4000 NTU stock suspension with high-purity water. *Standard Methods*, 19th edition, 1995, also describes procedures for directly preparing a stock suspension equal to 4000 NTU and a standard suspension (by dilution) of 40 NTU. *Appendix II* contains complete procedures, with dilution ratios,

for preparing turbidity calibration standards from 2 to 200 NTU from an initial dilution of 1000 NTU. Turbidity standards below 2 NTU are difficult to prepare accurately because the dilution ratio is so high that even minute variations in the volumes of formazin used and dilution water quality are significant.

Note that there is no sure way of producing or obtaining absolutely pure, turbidity-free water to use for dilutions. Multiple distillations, deionization, and ultrafiltration can all leave some residual particulate contamination, which will contribute to the turbidity of the final dilution. However, it is possible to account for low levels of dilution water turbidity by taking readings over a range of dilutions and extrapolating back to the pure dilution water value. Thus, given an initial dilution water turbidity less than 0.5 NTU, quite accurate low-level turbidity measurements can be made without the need to prepare extremely low-level calibration standards. Currently available turbidimeters are capable of automatically determining and compensating for initial dilution water turbidity values less than 0.5 NTU during the calibration procedure.

### Storage of Formazin Stock Suspensions

Chemical stability of formazin suspensions, like that of many other chemicals, is highly dependent on storage conditions. Exposure to heat or direct sunlight will break down the polymer structure, while prolonged exposure to ambient air allows the suspending fluid to evaporate and air-borne contaminants to enter. Store formazin standards in a cool, dark place. Refrigeration will provide extended life, but is not absolutely essential. Once a formazin standard has been removed from its

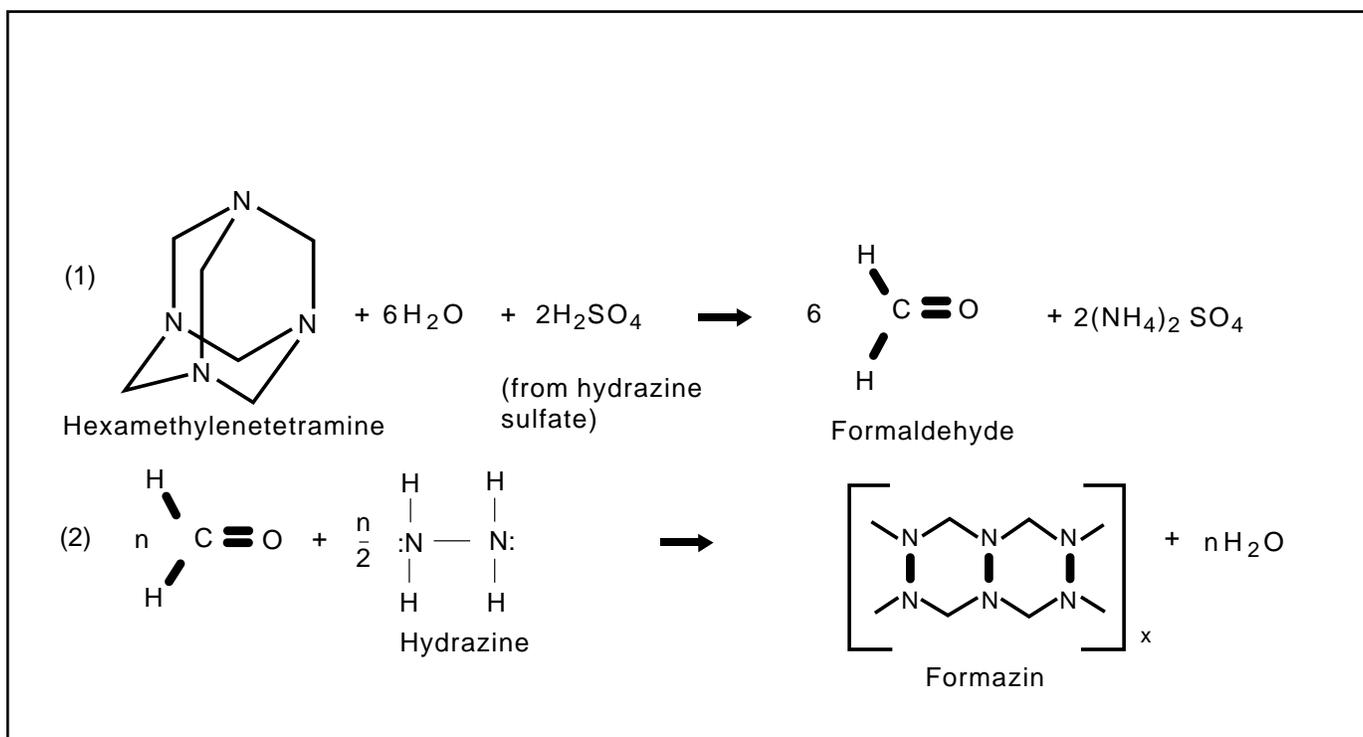


Figure 3: Preparation of formazin

storage bottle and used, it should be discarded. Do not leave containers open longer than necessary, and seal them tightly for storage. The pouring of these standards back into their storage containers will introduce contamination and could lead to erroneous future calibrations.

Properly stored, a 4000 NTU formazin suspension will be stable for about one year. Dilute formazin suspensions used as working standards are considerably less stable. As a rule the lower the concentration of a formazin standard, the lower the stability of that standard.

## StablCal Stabilized Formazin Standards

A new turbidity standard has been developed for use in calibrating or verifying the performance of any turbidimeter. StablCal Stabilized Formazin Standards contain the same light scattering polymer as traditional formazin primary turbidity standards. By using a different matrix, the polymer in StablCal standards will not deteriorate over time as is the case with low turbidity formazin standards. Due to this enhanced stability, StablCal standards of any concentration ranging up to 4000 NTU can be manufactured and packaged in ready-to-use formats. Thus, time is saved and direct exposure to the standard is minimized.

In trying to find a new or improved turbidity standard that would replace formazin, the following requirements for this standard must be met:

1. The standard should be stable for a long period of time.
2. The standard should be safe to handle.
3. The standard should be easy to prepare and to use.
4. The standard should have a particle size distribution that will encompass most water samples.
5. The standard should provide the same light scatter characteristics that closely resemble that of traditional formazin.
6. The standards should not be instrument specific. For example, a 2 NTU standard should read 2 NTU on any instrument regardless of make or model. These standards should be able to be interchanged with freshly prepared formazin standards of the same NTU value.
7. Preparation of the standard should be reproducible when using assayed starting materials. Accurate measurement apparatus and techniques must also be used. The preparation of these standards should be demonstrated to be repeatable.
8. Volumetric dilutions of a concentrated standard should be accurate and linear.

Several different materials were tested to determine if they met these requirements. Research into the area of finding a suitable replacement for formazin has been met with limited success. Formazin turbidity standards met many of these requirements, but still lacked the major feature of stability (particularly at low turbidity levels).

StablCal Stabilized Formazin Standards do meet all of these criteria. Two of the most important criteria are that the standards must be stable over time, regardless of concentration, and the standards provide comparable calibration response on any turbidimeter. Research that led to the development of the StablCal standards focused on these two areas of improvement over traditional formazin standards.

The development of a new stable turbidity standard (StablCal) is described below. This is followed by the comparability study performed after the stability was proven.

**Stability Research**—The key to stabilizing dilute formazin solutions is based on two points: Le Chatelier's Principle and proper standard dilution. Le Chatelier's Principle states: When more reactant is added to, or some product is removed from, an equilibrium mixture, thereby changing the concentration of the reactant or product, the net reaction will move from left to right to give a new equilibrium and more products being produced. In applying this principle to stabilizing formazin standards, it is known that there is an equilibrium that forms between reactants and products because residual hydrazine, the theoretical limiting reagent in the condensation reaction to produce the formazin polymer, is still present in parts per million amounts. This indicates that this reaction does proceed to near completion (>99%) but not to full completion. Formazin standards of low turbidity have the products removed through dilution. When a dilution is made, the equilibrium shifts back to the left toward the reactants and away from the products. Thus, the formazin polymer is more likely to degrade in order to compensate for this equilibrium shift. (The mechanism of this degradation is thought to be hydrolysis of the formazin polymer from the residual protons in solution.) In order to stabilize the formazin polymer, the reaction equilibrium needs to be pushed to the right as far as possible.

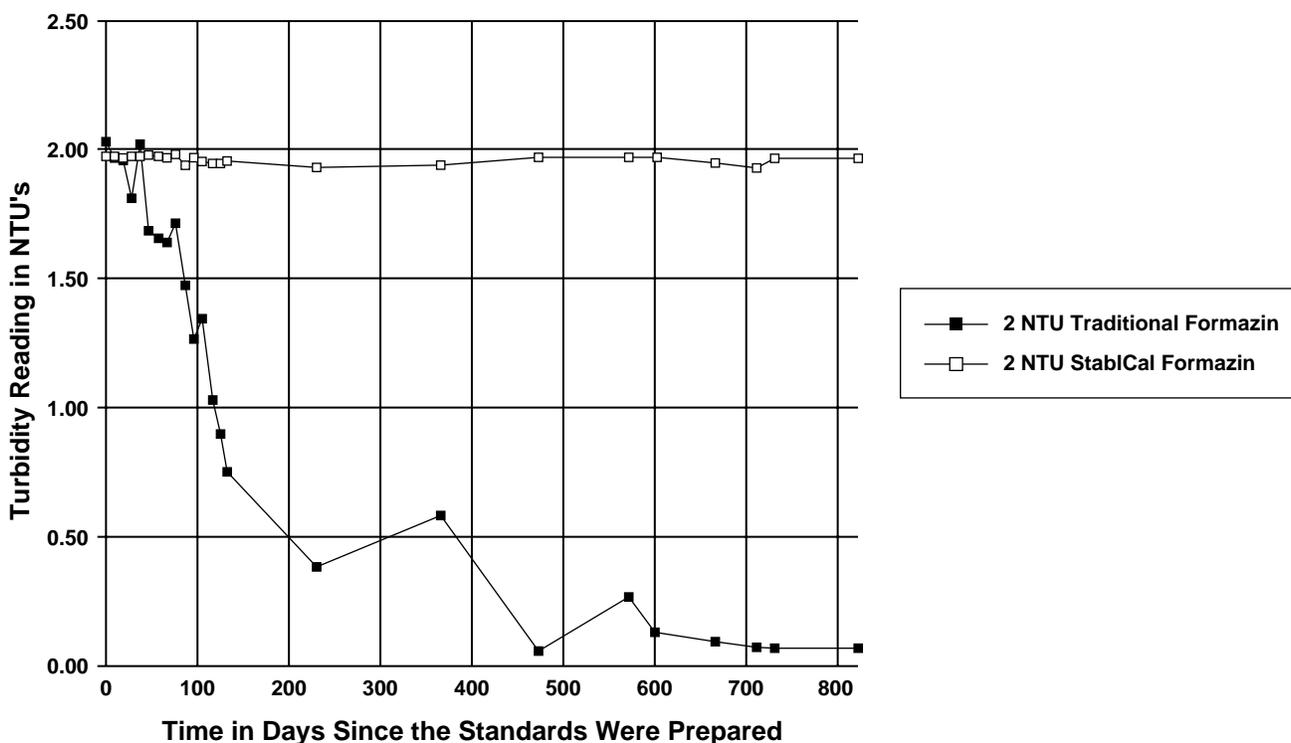
The second point to stabilizing the formazin polymer was based on changes that are made to the polymer and the matrix relative to a dilution. When a stock standard is diluted, the dilution is traditionally performed using ultrapure water. During this dilution, both the turbidity-causing polymer and any other components in the matrix are diluted. Theoretically, only the turbidity-causing formazin polymer should be diluted. The concentrations of all other components in the matrix should remain unchanged. Thus, when a concentrated formazin stock solution is traditionally diluted to make a low-turbidity standard using ultrapure dilution water, all components in the matrix are also diluted. The

equilibrium then shifts slightly back to the left, and the products are less stable. Thus, the matrix of a formazin standard is significantly different in a low-turbidity standard versus the highly concentrated 4000 NTU stock standard.

To verify this theory, some low turbidity formazin standards were prepared using two different procedures. In the first procedure, a 2-NTU standard was prepared using traditional techniques and ultrapure dilution water was used as the standard diluent. In the second procedure, a 2-NTU formazin standard was prepared using the same traditional techniques, but dilutions were performed using an ultra-filtered solution that replicated the matrix present in the stable 4000 NTU formazin concentrate. The standards were then monitored for stability for over 2+ years. This stability data is summarized in the graph in the table on the right and in Figure 4. This graph shows the 2-NTU standard prepared using dilution water remained stable for only a brief period of time. However, the other standard, prepared using the ultra-filtered solution that replicated the 4000 NTU matrix, continued to remain relatively unchanged throughout the duration of the test. This standard is the StablCal Stabilized Formazin Standard.

Time in Days Since The Standards Were Prepared	2 NTU Traditional Formazin	2 NTU StablCal Formazin
0	2.03	1.96
8	1.95	1.96
16	1.94	1.95
24	1.80	1.96
31	2.02	1.96
41	1.66	1.97
55	1.64	1.96
65	1.62	1.95
73	1.70	1.97
84	1.46	1.92
94	1.27	1.96
105	1.35	1.94
120	1.03	1.93
133	0.88	1.93
141	0.75	1.94
233	0.39	1.90
369	0.58	1.91
478	0.05	1.96
576	0.27	1.96
602	0.13	1.96
671	0.09	1.93
716	0.08	1.91
734	0.08	1.95
827	0.07	1.95

**Figure 4: Stability Comparison Between Turbidity Standards Prepared at 2 NTU— Traditional Formazin and StablCal Stabilized Formazin**



**Comparability:**

Once the StablCal standards were demonstrated to be stable, the next test was to determine and compare the functional aspects of StablCal standards to traditional prepared formazin standards.

The comparability research involved determining the performance of several turbidimeters that had been calibrated on StablCal standards to other formazin turbidity standards that had been prepared using traditional methodologies. The testing involved several makes and models of instruments. These instruments were calibrated on StablCal standards in the reporting range of 0 to 40 NTU. Then, fresh formazin standards were prepared from a primary stock standard concentrate using ultrapure water as the diluent. The traditional formazin and StablCal Standards were both read versus the StablCal based calibration on each instrument. The data was collected into Table 1.

Table 1 shows the instrument performance when using StablCal Stabilized Formazin Standards for calibration. The instruments accurately performed to within 1 percent of the theoretical value of the standards that were read in this study. This accuracy was based on several instruments, which further enhances the instrument-to-instrument comparability and accuracy of StablCal standards. Since the particle distribution of the StablCal Stabilized Formazin Standards matches that of formazin, the standard has a universal usage. Any standard can be used on any turbidimeter or nephelometer. Instrument-specific StablCal standards are not necessary.

**Table 1: Instruments calibrated on “traditional” formazin. Both StablCal™ Stabilized Formazin Standards and traditional formazin standards were read relative to “traditional” formazin. The results are displayed below:**

Instrument Make and Model	40 NTU StablCal Formazin	40 NTU Standard Formazin	20 NTU StablCal Formazin	20 NTU Standard Formazin	10 NTU StablCal Formazin	10 NTU Standard Formazin	5 NTU StablCal Formazin	5 NTU Standard Formazin	2 NTU StablCal Formazin	2 NTU Standard Formazin	1 NTU StablCal Formazin	1 NTU Standard Formazin
Hach 2100AN (NonRatio Mode)	39.7	39.4	20.1	19.9	10.0	9.91	5.15	5.02	2.03	2.07	1.10	1.05
Hach 2100AN Ratio Mode	40.0	40.3	19.7	19.9	9.78	10.1	4.94	5.00	1.97	2.06	1.07	1.03
Hach 2100AN IS NonRatio Mode	39.6	39.5	19.9	19.7	9.95	9.76	5.15	4.99	2.00	2.06	1.06	1.04
Hach 2100AN IS Ratio Mode	39.8	40.3	19.7	19.7	9.80	9.89	5.03	4.98	1.97	2.06	1.06	1.03
Monitek Model 21	38.3	39.0	19.7	19.8	9.90	9.87	4.97	4.83	1.95	1.97	1.05	1.01
LaMotte Model 2008	41.0	40.4	20.0	20.0	10.1	10.1	4.78	5.00	2.00	2.04	0.96	1.00
Hach 2100A	41.5	39.6	20.0	19.5	9.95	10.0	5.00	5.12	2.20	2.15	1.10	1.08
Hach 2100P	39.0	40.3	19.4	19.8	9.70	10.1	4.94	5.04	1.97	2.08	1.09	1.07

## Conclusion:

The StablCal Stabilized Formazin Standards have been shown to be both stable and read comparable to traditional, freshly prepared formazin standards. Standards in the range of 0.30 to 4000 NTU have been demonstrated to remain within 5 percent of their original preparation values for a minimum of two years. From a comparability standpoint, StablCal Standards can be interchangeably used as calibration standards on any turbidimeter with only very minimal differences in the instrument response.

The stabilization of formazin has resulted in the development of the StablCal standards. These standards serve as the solutions to those problems that were associated with traditional formazin standards. This stabilization allows for these standards to be packaged in structures that greatly reduce any kind of potential exposure to the user from the standard. Further, when comparing StablCal to traditional formazin standards of equal concentration, studies have shown that residual hydrazine sulfate in StablCal is reduced by two to three orders of magnitude. The stabilization of formazin in the StablCal standards provides the user with ready-to-use standards, and the large quantity of time required to prepare low turbidity traditional formazin standards is now eliminated. Users can take these stabilized standards and use them in the field, and at the same time be confident that the standards are accurate and repeatable in these non-laboratory settings.

## Secondary Standards

### Definition of Secondary Standard

Primary standards may be prepared directly, and their values are defined. The value of a secondary standard is derived by reference to a primary standard. By measuring against a primary standard, and in many cases adjusting to match the primary, a secondary standard is established as having a “known” value to be used for subsequent comparisons.

In physical measurement, secondary standards are required because there may be only one master standard defined and accepted as the standard unit (e.g. kilogram), and copies must be made for actual use. These secondary standards are often referred to as transfer standards, and are usually certified. Their derived value is traceable to the original primary standard. It is accepted that copies cannot be made to absolute perfection and contain probability for error.

In turbidity measurement, anyone with the proper materials and equipment can directly synthesize the primary standard. In this case, the need for secondary standards is more a matter of convenience, due to the instability of dilute formazin suspensions and the need to prepare fresh formazin dilutions for each use. **Secondary standards are used to check instrument calibration stability and are not to be used to perform a calibration.**

## Application of Secondary Turbidity Standards

Hach's secondary turbidity standards are particulate suspensions formulated to give results that match or read within a specified turbidity range. The value of a secondary standard is defined by comparison with a dilute formazin suspension. These types of secondary standards are used to verify instrument performance between primary standard calibrations, providing a stable reference and eliminating the need to prepare fresh formazin dilutions for routine calibration checks. These standards are not to be used to perform calibrations.

### Gelex® Secondary Turbidity Standards

Gelex secondary turbidity standards are a recent innovation in the field of turbidity measurement. Gelex standards are suspensions of metal oxide particles in a rubber-like polymer gel. Locked in place by the gel, the particles cannot settle, drift, or coagulate. The gel medium also protects the metal oxide particles from additional oxidation or contamination.

With care, Gelex secondary turbidity standards offer a nearly indefinite shelf life and very stable turbidity value. For many years, Hach provided secondary standards made from suspensions of polystyrene latex spheres. Because their shelf life was limited to about a year, Hach discontinued production of latex standards in favor of Gelex standards.

### Using Gelex Turbidity Standards

The light-scattering properties of any particle are a function of its size and shape, its refractive index, and the wavelength of the incident light. Different turbidimeters, using different light sources, detectors, and optical configurations, will respond differently to any standard other than formazin, the standard to which they've been calibrated. Gelex secondary standards, not having exactly the same light-scattering characteristics as formazin, must be specifically formulated for the turbidimeter model with which they will be used. (*Understanding Turbidity Science, Technical Information Series Booklet No. 11*, published by Hach Company, contains additional information about light scatter.)

Each Gelex secondary standard is supplied in an individual glass vial, and is formulated to provide a nominal turbidity value for a specific turbidity range on a specific Hach turbidimeter model. Turbidity ranges are marked on the individual vials. Actual values will vary slightly due to variations in glass vials and individual instruments. All secondary standards must be assigned a value on the turbidimeter with which they will be used, immediately after the instrument has been calibrated using formazin primary standards. The assigned value should be marked on the vial for use in verifying instrument calibration in day-to-day use. The Gelex standards should be re-measured each time the instrument is recalibrated with formazin, and new values reassigned as necessary.

## Care and Storage of Gelex Standards

Care should be taken in handling Gelex standards to avoid scratching the glass vial. While the stability of the gel medium minimizes the effects of heat, light and vibration on shelf life or value, it is important to protect Gelex standards from temperature extremes. Exposure to freezing or to temperatures greater than 50 °C (122 °F) for long periods may alter or damage the standards.

## Alternative Standards

### Definition of Alternative Standard

Primary formazin standards are accepted by the USEPA for turbidimeter calibration when results are being reported. Secondary standards such as Gelex, defined by comparison with formazin, are then used to verify calibration in day-to-day use. An alternative type of standard has recently been accepted by the USEPA for calibration purposes when reporting results. Currently available alternative standards cannot be prepared directly, requiring special facilities and controls, and still must be compared and traced to primary formazin during production in order to determine or adjust their value.

### Application of Alternative Standards

Alternative standards provide convenience in field use or other situations where the preparation of formazin dilutions is not practical. They are stable at low turbidity values and can be used for both calibration purposes and day-to-day verification.

Current alternative standards use a blend of styrene divinylbenzene (SDVB) cross-linked copolymer microspheres suspended in an ultrapure, aqueous medium. The spheres range from about 0.1 to 0.3 microns in diameter with a mean diameter of about 0.2 microns. This size distribution is narrower than that found in formazin (0.01 to 10.0 microns) and the particles are uniform spheres, so light-scattering properties and refractive characteristics are not the same as formazin. Alternative standards are adjusted during manufacture by comparison with formazin on the specific instrument for which they are prepared, and must be considered both instrument- and calibration-point specific. That is, both formazin measurements and alternative standard measurements will be within accuracy specifications for the values used as calibration points on the instrument for which they are intended, when the accuracy of the instrument, formazin standard, and alternative standard are all taken into consideration.

### Care and Storage of Alternative Standards

Alternative SDVB standards come in a range of NTU values and are ready for use. They may be transferred directly from bottle to sample cell if normal precautions are taken to avoid contamination. Never dilute alternative standards

and never pour material back into the bottle. Never freeze or boil. In addition, standards should be kept away from direct heat. Refrigeration is not recommended.

**Hach Company does not guarantee that performance specifications for its turbidimeters will be met if SDVB standards are used for calibration. Specifications are guaranteed only if freshly prepared formazin or StablCal Stabilized Formazin Standards are used for calibration.**

## Appendix I. Preparation of Primary Formazin

Formazin is an aqueous suspension formed by the reaction between hydrazine sulfate and hexamethylenetetramine. A 4000 NTU suspension is prepared by combining specified solutions of both and allowing the mixture to stand for 48 hours at 25 °C. Under the correct conditions, a 4000 NTU formazin suspension can be reproduced with a high degree of accuracy ( $\pm 1\%$ ) and is stable when properly stored.

Dilutions of the 4000 NTU stock suspension are less stable than original stock. Properly stored, a 4000 NTU suspension will last at least two years. A 400 NTU dilution is stable for only about one year. Hach chemists strongly suggest that working dilutions be prepared from a 4000 NTU stock suspension, used immediately, and then discarded. (See *Appendix III* for a complete listing of supplies available from Hach for preparing formazin.)

### Storage of Formazin Suspensions

The chemical stability of a formazin suspension is highly dependent on storage conditions. Exposure to heat or direct sunlight and prolonged exposure to ambient air can degrade shelf life significantly. Heat and light will break down the polymer structure, while exposure to air allows the suspending fluid to evaporate and air-borne contaminants to enter.

Store in a cool, dark place. Do not store above 40 °C. Refrigeration will provide extended shelf life but is not essential. Do not leave containers open longer than necessary and seal them tightly for storage. Allow formazin standards to acclimate back to room temperature before being used to calibrate.

### Formulating 4000 NTU Formazin Stock Suspension

**1.** Dissolve 1.000 g of ACS grade hydrazine sulfate,  $N_2H_4 \cdot H_2SO_4$ , in ultra filtered deionized water and dilute to 100 mL in a Class A, 100 mL volumetric flask. The hydrazine sulfate should be tested for purity, and only used if it is >99% pure.

**2.** Dissolve 10.00 g of analytical grade hexamethylenetetramine,  $(CH_2)_6N_4$ , in ultra-filtered deionized water and dilute to 100 mL in a Class A, 100 mL volumetric flask.

3. Quantitatively combine the equal volumes of the hydrazine sulfate solution and the hexamethylenetetramine solution into a clean, dry flask and mix thoroughly. Let the mixture stand for 48 hours at  $25 \pm 1$  °C. A white polymer suspension will form in the solution during this period. The turbidity of this primary formazin suspension is 4000 NTU. Store this suspension in a bottle that filters ultraviolet light.

The recommended storage for formazin is between 5 and 25 °C. Allow the standard to come to equilibrium temperature and mix well before use.

## Appendix II. Preparing Formazin Dilutions

1. Using a 10.0 mL TenSette® Pipet, prepare a 1000 NTU formazin working suspension by transferring 25 mL of 4000 NTU formazin stock suspension into a 100 mL volumetric flask\*. Dilute to the mark with turbidity-free water; swirl to mix. See notes A, B, C, and D.

2. Using a 10.0 mL TenSette Pipet or a 1.0 mL TenSette Pipet, transfer the desired amount of the 1000 NTU working suspension to a 100 mL volumetric flask. Dilute to the mark with turbidity-free water and mix.

3. Rinse a clean turbidimeter sample cell once with the prepared formazin standard, then carefully pour the appropriate volume of standard into the cell. Insert the cell into the turbidimeter and calibrate according to instructions in the turbidimeter manual.

The required amount of 1000 NTU working suspension to be used may be determined by using the formula:

$$\frac{(\text{volume of dilution to be prepared}) \times (\text{desired NTU value})}{(\text{NTU of working suspension})} = \text{required amount}$$

For example, to prepare a 100 mL, 4 NTU suspension starting with a 1000 NTU working suspension, find the required amount of 1000 NTU suspension:

$$\frac{(100 \text{ mL}) \times (4 \text{ NTU})}{(1000 \text{ NTU})} = 0.4 \text{ mL}$$

To prepare the working standard, transfer 0.4 mL of 1000 NTU suspension to a flask and dilute to 50 mL with ultra-low turbidity water.

Table 2 shows the amounts of 1000 NTU initial dilution required to prepare 100 mL suspensions for a range of working standards.

Table 2

Desired NTU Standard	mL of 1000 NTU Suspension
2	0.2
4	0.4
6	0.6
10	1.0
20	2.0
40	4.0
100	10.0
200	20.0

### Notes

**A.** Dilute formazin standards are stable only for a short period of time, and should be used immediately after preparation.

**B.** Use Hach prepared 4000 NTU Formazin Stock Suspension, or prepare a 4000 NTU suspension following the procedure in Appendix I.

**C.** Turbidity-free water for measuring turbidities as low as 0.02 NTU may be prepared by filtering distilled water through a 0.2-micron membrane filter or through a reverse osmosis filtration unit. High quality demineralized water may also be used as long as its turbidity is below 0.1 NTU.

**D.** Mix turbidity standards by swirling or gently inverting. Shaking may introduce bubbles and cause erroneous readings. Vigorous shaking will also introduce oxygen and cause oxidation of the formazin polymer chains, and will also cause fracturing of the polymer chains.

## Appendix III. Preparing and Using StablCal Stabilized Formazin Standards:

- Do not transfer the standard to another container for storage.
- Store standards between 5 and 25 °C. Avoid prolonged exposure to temperatures exceeding 25 °C.
- Do not pour standard from the sample cell back into its storage container (Bulk Solutions).
- For long-term storage, refrigeration at 5 °C is recommended. Do not store above 25 °C.
- Always allow the standard to acclimate to ambient instrument conditions before use (not to exceed 40 °C).
- Store away from direct sunlight. Vials should be stored in their respective kit with the cover on.
- For all StablCal standards greater than the diluent solution, mix the standard well before transferring to a sample cell. Never mix the diluent solution.

\* For greatest accuracy, procedures may be performed with Class A volumetric glassware.

Instructions are for two conditions. Follow the first set of instructions if your standards have been sitting undisturbed for longer than one month, or if you need to prepare your standards. Follow the second set of instructions if the standards have been prepared (transferred into vials) and are used frequently.

### **Preparing StablCal Standards (After Long-Term Storage):**

(Bulk or Vial Standards):

*Note: If using bulk standards, perform step 1 (below) before breaking the seal on the bottle. If your standards have been sitting undisturbed for longer than one month, shaking the standard will breakup the condensed suspension into its original particle sizes.*

*Note: If you are using the <0.1 NTU StablCal Solution, do not mix. Follow the instructions for using <0.1 NTU StablCal solution.*

1. Shake the standard vigorously for 2 to 3 minutes.
2. Allow the standard to stand for 5 minutes.
3. Eliminate any optical variances in the sample cell using traditional preparation techniques (oiling and indexing the sample cell).
4. For bulk solutions, invert at least 50 times before pouring the standard into the sample cell. Rinse the cell at least one time with the bulk standard.
5. Fill the sample cell with the standard; cap the sample cell and, if used, cap, the bulk sample.
6. Invert the sample cell two to three times; let the sample sit undisturbed for two to three minutes.
7. Place the sample cell into the turbidimeter and record the value, or perform the calibration point measurement.

### **Using Prepared StablCal Standards (When Used Frequently):**

(Bulk or Vial Standards)

*Note: If you are using the <0.1 NTU StablCal Solution, do not mix. Follow the instructions for using <0.1 NTU StablCal solution.*

1. Invert the standard a couple of times to ensure a homogeneous solution.
2. Eliminate any optical variances in the sample cell using traditional preparation techniques (oiling and indexing the sample cell).
3. For bulk solutions, invert a couple of times before pouring the standard into the sample cell. Rinse the sample cell at least one time with the bulk standard.
4. Wait 2 to 3 minutes.
5. Place the standard into the turbidimeter; record the value or perform the calibration point measurement.

### **Using the <0.1 NTU StablCal Solution:**

(Bulk or Vial Standards)

**IMPORTANT: Substitute this standard for the dilution water when performing a calibration using the StablCal Standards.**

*Note: If the <0.1 NTU StablCal Solution has recently been mixed, let stand 10 to 15 minutes before using.*

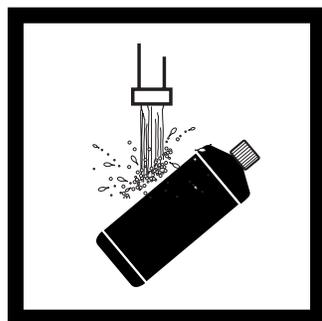
1. Do not shake or invert. Do not generate any bubbles in solution. Let the standard sit undisturbed for one to 5 minutes before use.
2. Carefully pour the standard down the inside wall of the sample cell; this prevents any air bubbles from forming in the sample cell.
3. Immediately cap the sample cell and standard bottle.
4. Wait 1 to 3 minutes.
5. Place the sample cell in the turbidimeter; record the value.

## Appendix IV. Using Low Range StablCal Standards for verification of calibration (Standards between 0.10 and 1.0 NTU)



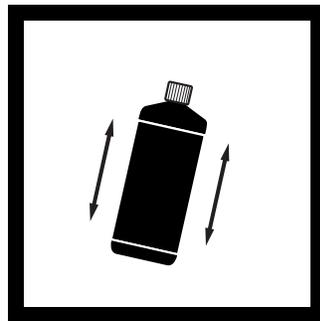
**1.** Thoroughly clean and rinse the sample cell.

*Note: For laboratory and portable instruments, see Note a. For a process turbidimeter, see Note b.*



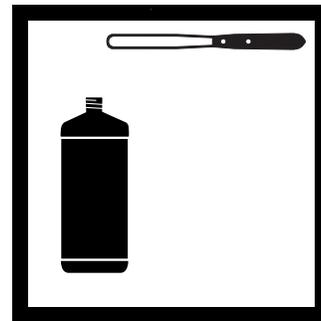
**2.** Rinse the outside of the StablCal Standard bottle with water. This removes any dust or particulate matter that could contaminate the standard. Dry the bottle with a lint-free cloth or tissue.

*Note: If necessary, allow the standard to come to ambient temperature before use.*



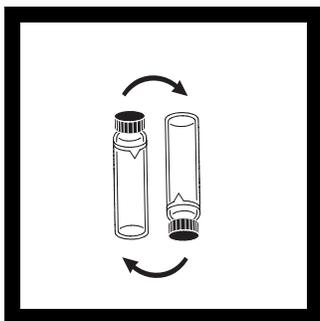
**3.** Gently invert the Low-Range StablCal Standard bottle at least 50 times to thoroughly mix and re-suspend the formazin polymer. See Note c.

*Note: Do not shake or invert rapidly. Entrained air bubbles and erroneous readings could result. See Note d.*

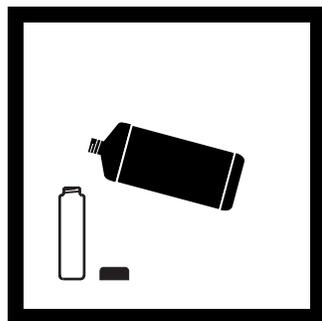


**4.** Using a clean knife, carefully remove the heat seal from the StablCal Standard bottle. After removing the heat seal, always keep the container tightly capped in order to minimize contamination.

*Note: Uncontaminated standard will retain its turbidity value for at least one month after removing the heat seal.*

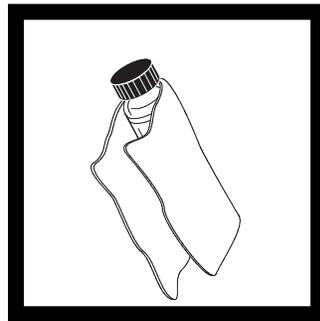


**5.** Fill a sample cell at least half full with StablCal Standard. Cap and invert the sample cell several times. Immediately discard the rinsings. Repeat this rinsing procedure at least one more time.

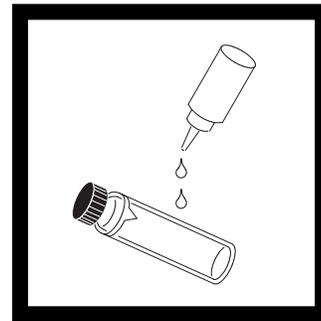


**6.** Slowly fill the sample cell with StablCal Standard. Immediately cap both the sample cell and the bottle of standard. See Notes e and f.

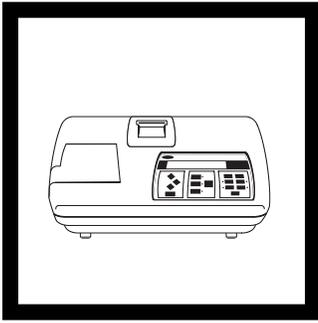
*Note: For a process turbidimeter, fill the turbidimeter body with standard and immediately re-position the turbidimeter bead to begin taking readings.*



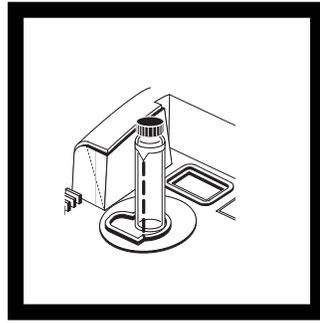
**7.** Wipe the cell with a soft, lint-free cloth to remove water spots and fingerprints.



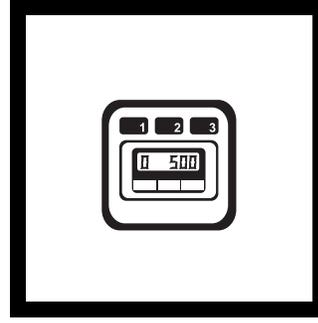
**8.** Apply a thin film of silicone oil to the sample cell. Wipe with a soft cloth to obtain a thin and even film over the entire surface.



**9.** Turn the instrument on. Allow it to warm up per the manufacturer's instructions.

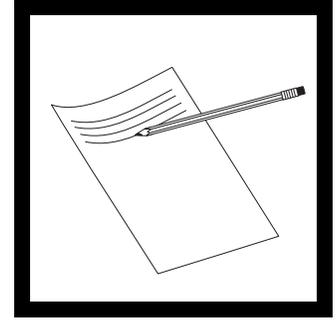


**10.** Place the sample cell in the instrument cell in the instrument compartment using the proper indexed orientation. Close the lid.



**11.** Wait at least 5 minutes to allow entrained bubbles to vacate the standard matrix. Take measurement between 5 and 60 minutes.

*Note: During the waiting period, the instrument's digital display reading may fluctuate. As bubbles dissipate from the standard, the display value will become more stable.*



**12.** Record the stable reading, which will remain unchanged (to the nearest 0.003 NTU) for at least 15 seconds.

*Note: Spikes can occur and are generally displayed as a sudden increase in turbidity, followed by a slower decrease back to the base value. Stray bubbles, uncharacteristically large particles, or electronic noise can cause spiking. Ignore spikes.*



**13.** The instrument accuracy is verified if the reading is within the tolerances on the StablCal Standard Certificate of Analysis. If the reading is outside of the stated specification for this test standard, see troubleshooting section.

## Troubleshooting

### Laboratory and Portable Instruments

For high readings, first inspect the sample cell for scratches, abrasions or flaws. Re-apply a thin and even film of silicone oil to the sample cell. Measure the standard again. If the reading is still high, clean the inside of the cell and rinse the cell several times with ultra-filtered deionized water. (Ultra-filtered water may be produced by filtering deionized water through a 0.2- $\mu$ m filter or smaller. This water will have a turbidity between 0.020 and 0.050 NTU.) Rinse the cell twice with the standard and repeat the measurement. If the turbidimeter still reads out of specification, then recalibrate the instrument.

### Process Instruments

If the process instrument reading is high, clean the turbidimeter body and bubble trap again. Rinse thoroughly with ultra-filtered deionized water. (Ultra-filtered water may be produced by filtering deionized water through a 0.2- $\mu$ m filter or smaller. This water will have a turbidity between 0.020 and 0.050 NTU.) Repeat the test with fresh standard. The previously used standard may have been contaminated from a dirty turbidimeter body and should not be reused.

### Notes

**a.** Use one of the following cleaning procedures for sample cells. The first procedure involves filling the sample cells with 1:1 hydrochloric acid. Cap the cells and place them in a sonic bath for 5 minutes. Then, allow the cells to stand for another 30 minutes. Follow by rinsing both the cells and caps at least 10 times with ultra-filtered deionized water. Immediately cap after rinsing in order to prevent contamination. Dry the outside surface of the capped cell with a soft cloth.

The second cleaning procedure involves scrubbing the sample cells, both inside and out, with a mild laboratory detergent (e.g., Liqui-nox® Detergent), and a soft brush. Immediately follow by rinsing at least 10 times with ultra-filtered water. Cap to prevent contamination. Dry the outside surface of the capped cell with a soft cloth.

**b.** For process instruments, both the bubble trap and the turbidimeter body must be thoroughly cleaned. Refer to the cleaning instructions in the respective instrument manual for details.

**c.** Once the StablCal Standard has been mixed, it has a reading window of between 5 and 60 minutes, excluding spikes. During this time, the standard does not need to be re-mixed. The standard should be inverted between 3 to 5 times every hour to keep the formazin polymer suspended.

**d.** Do not use a sonic bath to degas the standard. Sonication will fracture micro-sized particles of glass into the standard, which will contaminate it.

**e.** These standards do not remain stable for long periods of time when continuously in contact with glass surfaces. At this level of turbidity, the standard can leach particulate matter from the glass back into the standard resulting in a slight increase in turbidity. Long-term stability requires storage in the original container. You should only leave the standard in a sample cell for up to one week or as experience dictates. Once the standard has been used, discard it.

**f.** Do not pour the standard back into its storage container or contamination of the standard will occur and future readings will be high.

### Stability and Shelf-Life of Ultra-Low StablCal Turbidities Standards

Long-term storage of StablCal Standards in glass sample cells is not recommended. The matrix of the ultra-low standards is so clean that small amounts of glass particulate matter can leach from the glass sample cell walls. This leaching will result in a mild increase in the turbidity of the standard. This leaching effect is only observed at ultra-low turbidity levels of <1.0 NTU. Above 1.0 NTU, there is enough formazin polymer present to reduce the aggressiveness of the matrix and reduce the leaching of the glass. In summary, if the sample cells are cleaned extremely well before use, StablCal Standards can remain in sample cells for approximately one week. If short-term storage in glass sample cells is required, special Teflon® lined caps must be used with glass sample cells. The Teflon caps prevent contamination and leaching into the standard.

## Appendix V. Apparatus and Reagents

Cat. No.	Description	Unit
742-46	Hydrazine Sulfate, ACS grade	20 g
742-26	Hydrazine Sulfate, ACS grade	100 g
1878-34	Hexamethylenetetramine, analytical grade	500 g
14574-42	Volumetric Flask, 100 mL, Class A	each
20858-56	Amber Bottle, Narrow Mouth, LPE, 250 mL, 6/pkg	each

### Prepared Formazin Stock Suspension:

2461-49	4000 NTU Formazin Stock Suspension	500 mL
2461-01	4000 NTU Formazin Cartridge for the Digital Titrator	each
16900-01	Digital Titrator	each
24018-12	Sample Cell Caps, Teflon lined, 12/pkg	pkg

### StablCal Vial and Bulk Solution Calibration Kits

26591-05	StablCal Vial Calibration Kit for the 2100A Turbidimeter	set
26592-05	StablCal Vial Calibration Kit for the Ratio XR and Ratio 2000 Turbidimeters	set
26593-05	StablCal Vial Calibration Kit for the Ratio Turbidimeter	set
26594-05	StablCal Vial Calibration Kit for the 2100P Turbidimeter	set
26621-05	StablCal Vial Calibration Kit for the 2100N Turbidimeter	set
26595-05	StablCal Vial Calibration Kit for the 2100AN and ANIS Turbidimeter	set
26595-05	StablCal Vial Calibration Kit for the 2100A Turbidimeter	set
26591-00	StablCal Bulk Solution Calibration Kit for the 2100A Turbidimeter	set
26592-00	StablCal Bulk Solution Calibration Kit for the Ratio XR and Ratio 2000 Turbidimeters	set
26593-00	StablCal Bulk Solution Calibration Kit for the Ratio Turbidimeter	set
26594-00	StablCal Bulk Solution Calibration Kit for the 2100P Turbidimeter	set
26621-00	StablCal Bulk Solution Calibration Kit for the 2100N Turbidimeter	set
26595-00	StablCal Bulk Solution Calibration Kit for the 2100AN and 2100AN IS Turbidimeter	set
26596-00	StablCal Bulk Solution Calibration Kit for the 1720C Turbidimeter	set
26598-49	StablCal Bulk Solution, 1.0 NTU	each
27146-00	StablCal Low Range Calibration Verification Kit (0.30, 0.50, 1.0 NTU, 100 mL, 1 each)	set
27146-04	StablCal Low Range Calibration Verification Kit (0.30, 0.50, 1.0 NTU, 100 mL, 4 each)	set
27163-00	StablCal Low Range Calibration Verification Kit for 1720C and 1720D Turbidimeters (0.30, 0.50, 1.0 NTU, 1000 mL, 1 each)	set

### For preparation of formazin dilutions, conventional glassware method:

14574-42	Volumetric Flask, 100 mL, Class A	each
14574-41	Volumetric Flask, 50 mL, Class A	each
14515-40	Volumetric Pipet, 25.00 mL, Class A	each
14515-34	Volumetric Pipet, 0.50 mL, Class A	each
14515-35	Volumetric Pipet, 1.00 mL, Class A	each
14515-36	Volumetric Pipet, 2.00 mL, Class A	each
14515-37	Volumetric Pipet, 5.00 mL, Class A	each
14515-06	Volumetric Pipet, 6.00 mL, Class A	each
14515-07	Volumetric Pipet, 7.00 mL, Class A	each
14515-08	Volumetric Pipet, 8.00 mL, Class A	each
14515-09	Volumetric Pipet, 9.00 mL, Class A	each
14515-38	Volumetric Pipet, 10.00 mL, Class A	each

### Or, in place of the 0.50 through 10.00 mL pipets, use TenSette Pipets:

19700-01	TenSette Pipet, 0.1 to 1.0 mL, 0.1 mL increments	each
19700-10	TenSette Pipet, 1.0 to 10.0 mL, 1.0 mL increments	each

### Gelex Secondary Standard Sets:

22526-00	For Hach Model 18900 Ratio Turbidimeter	set
22956-00	For Hach Model 2100A Turbidimeter*	set
22958-00	For Hach Model 16800 PortaLab Turbidimeter	set
23287-00	For Hach Ratio/XR and Ratio 2000 Turbidimeter	set
24641-05	For Hach Model 2100P Portable Turbidimeter	set
25890-00	For Hach Model 2100N Laboratory Turbidimeter	set
25892-00	For Hach Model 2100AN Laboratory Turbidimeter	set

\* Between regular calibration with formazin primary standards, Gelex secondary standards may be used to adjust instrument calibration before use.