# *Fluorescein NIST-Traceable Standard (F36915)*

## Quick Facts

***Storage upon receipt:***

- ≤6ºC
- Protect from light

## Introduction

Molecular Probes is the exclusive manufacturer of the fluorescein solid used in Standard Reference Material 1932 (SRM® 1932, National Institute of Standards & Technology (NIST)). This certified fluorescein in solution at pH 9 meets the needs of both research and industry for a reference standard for fluorescence intensity. Molecular Probes' fluorescein NIST-traceable standard solution (F36915) is prepared using fluorescein and buffer that meet the strict criteria established by NIST, and is directly traceable to SRM 1932. The fluorescein standard can be used as a reference material for calibrating instruments or for determining Molecules of Equivalent Soluble Fluorophore (MESF) values for experimental solutions.

Molecular Probes' fluorescein NIST-traceable standard is a calibrated 50 μM solution of fluorescein in 100 mM sodium borate buffer, pH 9.5. At pH 9.0 the dianionic fluorescein is strongly fluorescent, exhibiting a quantum yield of 0.93. At lower pHs, fluorescein can exist in multiple ionization states (Figure 1), with $pK_a$s of 2.2, 4.3 and 6.4 for the cation/lactone (neutral), the lactone/monoanion and the monoanion/dianion transitions, respectively.[1] For example, at pH 7.2, fluorescein is 91% dianionic and 9% monoionic. Both the absorbance and fluorescence emission of fluorescein are affected by pH (Figure 2). Thus, when the reference solution is to be used to determine the concentration of an unknown solution by fluorescence, it is critical to match the pH of the two solutions, e.g. by using the same buffered diluent and using sufficiently high dilution factors.

The fluorescein NIST-traceable standard can be used to establish MESF units for an unknown sample. MESF units take into account differences that may occur between the fluorescence of the analyte and the reference solution, if the two materials are analyzed on the same instrument with exactly the same conditions.[2,3] MESF values do not indicate the number of molecules but rather the equivalent number of fluorophores dissolved in the same medium, regardless if they are free in solution, attached to microspheres, nucleic acids or antibodies.[2,4]

### Material

The fluorescein NIST-traceable standard is supplied as a set of five vials, each containing 1 mL of fluorescein at a nominal concentration of 50 μM, in 100 mM sodium borate buffer at pH 9.5. The exact concentration of the solution is lot specific and is denoted on the vial and in the Certificate of Analysis.

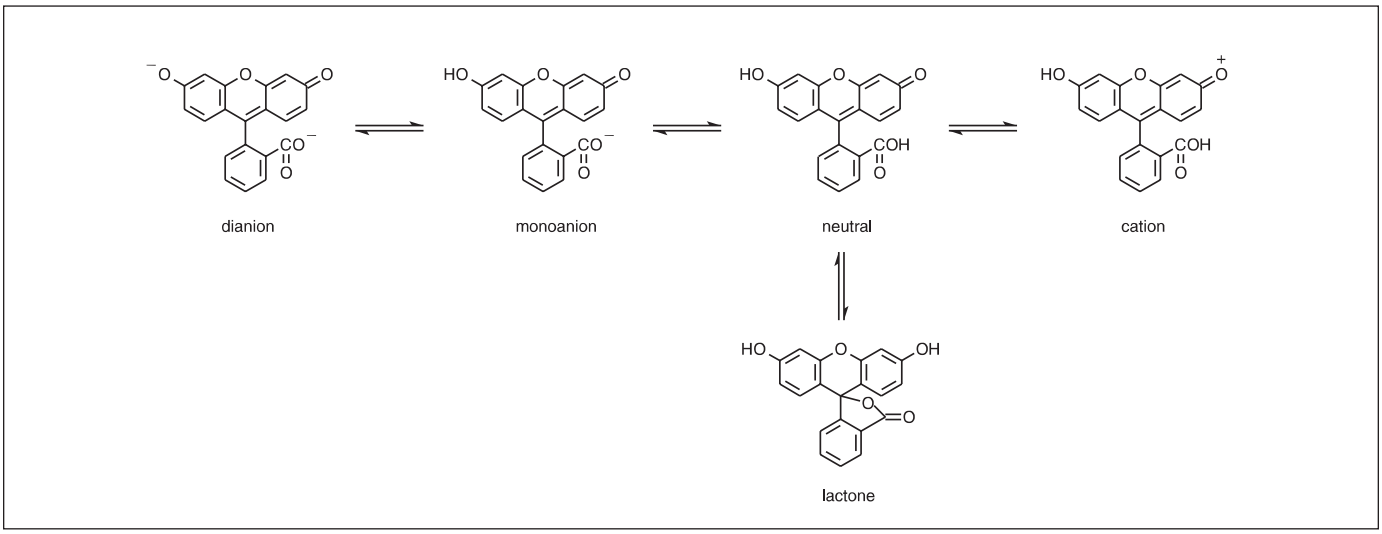


**Figure 1.** Ionization equilibria of fluorescein.

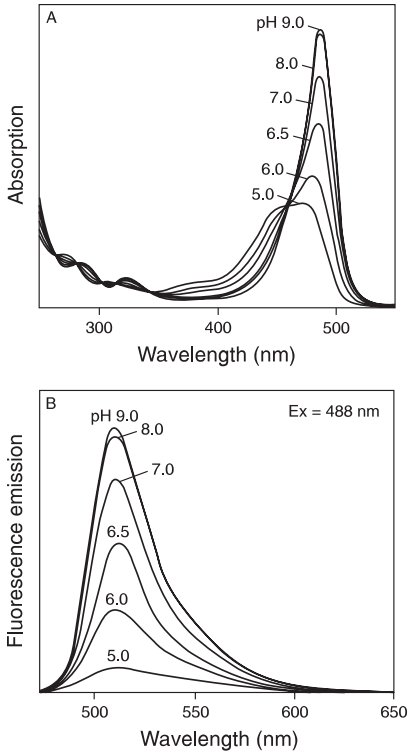


**Figure 2.** The pH-dependent absorption and fluorescence emission spectra of fluorescein.

## Storage and Handling

Upon receipt, store the fluorescein NIST-traceable standard at ≤6°C protected from light. The solution should be stable for at least 1 year from the date of purchase when stored properly. Prepare working solutions immediately before use, and then discard the working solutions after use.

## Application

Because the actual fluorescence units can vary as a function of several factors including pH, temperature and instrument settings, it is important to rigorously control these variables. For example, dilute the standard with the same buffer as that used with the analyte of interest and use exactly the same instrument settings. A 100-fold dilution of the standard solution is recommended as a starting point before measuring fluorescence.

## References

**1.** J Fluorescence 6, 147 (1996); **2.** J Res Natl Inst Stand Technol 106, 381 (2001); **3.** J Res Natl Inst Stand Technol 107, 339 (2002); **4.** J Res Natl Inst Stand Technol 107, 83 (2002).

## Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

| Cat # | Product Name | Unit Size |
|---|---|---|
| F36915 | fluorescein *NIST-traceable standard* *nominal concentration 50 µM* *special packaging* | 5 x 1 mL |

## *Contact Information*

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information.


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