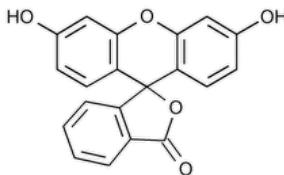


Status: Currently Official on 14-Feb-2025  
 Official Date: Official as of 01-May-2020  
 Document Type: USP Monographs  
 DocId: GUID-11417500-20BA-4E3D-BA3A-A3B7319AA1FA\_2\_en-US  
 DOI: [https://doi.org/10.31003/USPNF\\_M33580\\_02\\_01](https://doi.org/10.31003/USPNF_M33580_02_01)  
 DOI Ref: eo5c2

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## Fluorescein



$C_{20}H_{12}O_5$  332.31

Spiro[isobenzofuran-1(3H),9'-[9H]xanthen-3-one, 3',6'-dihydroxy-.

Fluorescein CAS RN®: 2321-07-5.

» Fluorescein contains not less than 97.0 percent and not more than 102.0 percent of  $C_{20}H_{12}O_5$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference Standards (11)**—

<a href="#">USP Diacetylfluorescein RS</a>	$C_{24}H_{16}O_7$	416.39
<a href="#">USP Fluorescein RS</a>		

**Change to read:**

**Identification**, ▲ [SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Infrared Spectroscopy: 197K](#)▲ (CN 1-May-2020) : previously dried over silica gel for 16 hours.

**WATER DETERMINATION, Method I (921)**: not more than 1.0%.

**Zinc**—Suspend 100 mg in 10 mL of a saturated solution of sodium chloride, add 2 mL of 3 N hydrochloric acid, mix, filter, and add 1 mL of potassium ferrocyanide TS to the filtrate: no turbidity is produced.

**Acriflavine**—Suspend 10 mg in 5 mL of water, swirl the mixture, and filter. To the filtrate add a few drops of sodium salicylate solution (1 in 10): no precipitate is formed.

### Assay—

**Standard preparation**—Dissolve about 110 mg of [USP Diacetylfluorescein RS](#), accurately weighed, in 10 mL of alcohol contained in a 100-mL volumetric flask. Add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 1.1 µg of diacetylfluorescein per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see [Buffer Solutions](#) in the section [Reagents, Indicators, and Solutions](#)), dilute with water to volume, and mix.

**Assay preparation**—Dissolve about 90 mg of Fluorescein, accurately weighed, in 10 mL of alcohol contained in a 100-mL volumetric flask. Add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Dilute quantitatively and stepwise with water to obtain a solution having a concentration of 0.9 µg per mL.

Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see [Buffer Solutions](#) in the section [Reagents, Indicators, and Solutions](#)), dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the fluorescence intensities,  $I$ , of the *Standard preparation* and the *Assay preparation* in a fluorometer at an excitation wavelength of 485 nm and an emission wavelength of 515 nm. Calculate the quantity, in mg, of  $C_{20}H_{12}O_5$  in the Fluorescein taken by the formula:

$$(332.31/416.39)(3333C)(I_u/I_s)$$

in which 332.31 and 416.39 are the molecular weights of fluorescein and diacetylfluorescein, respectively;  $C$  is the concentration, in µg per mL, of [USP Diacetylfluorescein RS](#) in the *Standard preparation*; and  $I_u$  and  $I_s$  are the fluorescence values observed for the *Assay preparation* and the *Standard preparation*, respectively.

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
FLUORESCEIN	<a href="#">Documentary Standards Support</a>	SM32020 Small Molecules 3

**Most Recently Appeared In:**

Pharmacopeial Forum: Volume No. 46(5)

**Current DocID: GUID-11417500-20BA-4E3D-BA3A-A3B7319AA1FA\_2\_en-US**

**DOI: [https://doi.org/10.31003/USPNF\\_M33580\\_02\\_01](https://doi.org/10.31003/USPNF_M33580_02_01)**

**DOI ref: [eo5c2](#)**

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