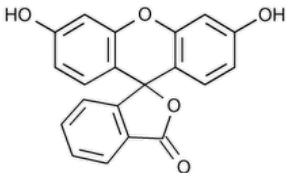


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# Fluorescein



$C_{20}H_{12}O_5$  332.31  
Spiro[isobenzofuran-1(3*H*),9'-[9*H*]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)**—

[USP Diacetylfluorescein RS](#)  $C_{24}H_{16}O_7$  416.39  
[USP Fluorescein RS](#)

**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<sub>u</sub>* and *I<sub>s</sub>* 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
FLUORESCIN	<a href="#">Documentary Standards Support</a>	SM32020 Small Molecules 3

Chromatographic Database Information: [Chromatographic Database](#)

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