

***Reagents Provided***

**Avidin-Fluorescein:** 1 mL of Avidin-FITC at a concentration of 10 µg/mL.

***Intended Use***

Used as a secondary reagent in immunofluorescent assays using biotinylated primary labeling reagents. This product has been optimized for use with monoclonal antibody-biotin reagents.

***Background Information***

Avidin is a glycoprotein of 66,000 daltons that is highly water soluble. This molecule is stable over a wide range of temperatures and pH. Its usefulness in immunoassays is largely derived from the fact that it can bind four biotin molecules for every molecule of avidin. The binding of avidin to biotin represent one of the strongest noncovalent ionic interactions with a dissociation constant of approximately  $1 \times 10^{-15}$  M.

***Reagent Preparation***

**Avidin-Fluorescein (Av-FITC)** is produced as the FITC conjugate of avidin at a molar ratio of FITC:protein of 5.2. Av-FITC is provided in a ready-to-use liquid format containing 0.15 M NaCl, 10 mM HEPES, 0.5% BSA, and 0.09% NaN<sub>3</sub>. The FITC molecule can be excited by laser light at 488 nm and has an optimal emission at wavelength at 525 nm. Store reagent at 2 - 8° C. **DO NOT FREEZE.** Reagent is stable for at least 6 months after purchase.

***Sample Staining***

10 µL of Avidin-FITC is added to a maximum of  $1 \times 10^6$  cells in 100 - 200 µL that have been optimally stained previously with a biotinylated primary reagent. The reaction is then allowed to proceed another 30 - 45 minutes at 2 - 8° C **in the dark**. The cell mixture is then washed twice with 10 mM PBS. The final cell pellet is resuspended in 200 - 300 µL of 10 mM PBS for flow cytometric analysis. Av-FITC stained cells should be kept in the dark if storage is required prior to flow cytometric analysis.

**N.B.** Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.