# RD SYSTEMS a biotechne brand

## Flow Cytometry Secondary Reagents

Donkey Anti-Sheep IgG (H+L)-Fluorescein Catalog Number: F0125 Lot Number: ABSW03 100 Tests

#### Intended Use

This reagent is designed for use as a secondary developing reagent in immunofluorescent assays, such as flow cytometry, where the primary antibody does not have a fluorescent reporter molecule, is of sheep origin, and is of the IgG class.

### **Background Information**

This polyclonal antibody preparation has been derived from donkey immunized with sheep IgG. Sheep IgG specific IgG is purified by sheep IgG (H+L) affinity chromatography and absorbed against human IgG, and human, mouse, bovine, and rabbit serum to eliminate cross-reactivity. The IgG fraction is then conjugated to fluorescein for use in immunofluorescent-type assays.

#### **Reagents Provided**

Supplied as 25  $\mu g$  of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

#### Storage

Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

### **Reagent Preparation**

**Donkey anti-sheep IgG (H+L)-Fluorescein** is produced as the fluorescein derivative of donkey IgG from animals immunized with sheep IgG. The reagent is provided in a ready-to-use liquid format containing phosphate buffered saline with 0.5% BSA and 0.1% NaN<sub>3</sub> as a preservative. Store reagent at  $2^{\circ}$  -  $8^{\circ}$  C. **DO NOT FREEZE**. Dispose of liquids containing azide with caution and according to local regulations.

#### Sample Staining

- Cells of interest (up to 1 x 10<sup>6</sup> cells) are stained with a sheep IgG primary antibody according to the antibody manufacturer's instructions.
- 2. After the recommended incubation period, the cells are washed 3 times with a PBS buffer followed each time by centrifugation at 250 x g for 5 minutes.
- The cell pellet is resuspended in up to 200 μL of PBS and 10 μL of donkey anti-sheep IgG (H+L)-CFS is added to each reaction.
- The cells are incubated for 30 minutes at 2° 8° C in the dark. The cells are washed 3 times as indicated in step # 2.
- 5. The cell pellet is resuspended in 400  $\mu$ L of PBS for flow cytometric analysis.

Warning: 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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.