Product No. K8010

Summary: American Qualex offers a complete kit for the quick and easy labeling of antibodies or other proteins with fluorescein (FITC). FITC-antibody conjugates are useful in many applications such as flow cytometry and immunocytochemistry. Direct labeling of the primary antibody eliminates the need for a second antibody and results in lower background and an overall higher Signal-to-Noise ratio. Other proteins such as steroids can also be labeled with the FITC Labeling Kit for easy detection.

Storage: Store refrigerated (2-8°C).

Materials Provided: Each kit includes complete protocols and sufficient reagents to perform 5 conjugations of 100-250 µg each:

- FITC: 5 x 0.85 mg
- Solvent Reagent -DMF: 5.0 ml
- 10X PBS Buffer Concentrate: 100 ml (contains 1% NaN_3)
- Carbonate Buffer Concentrate: 100 ml
- NEW now provided Sodium Chloride 10 x 8.7 grams
  (1 bottle for each liter of Carbonate Saline Buffer)

Materials Required But Not Provided: The following materials are required but are not provided in the kit:

- Sodium Azide (NaN_3)

  This item is optional. Some antibodies may precipitate in the absence of sodium chloride, especially monoclonals. No adverse effects have been seen with the routine addition of sodium chloride.

920-A Calle Negocio San Clemente California 92673
(949) 492-8298 (800) 772-1776 FAX: (949) 492-6790
E-mail: info@americanqualex.com
www.aqsp.com

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American Qualex Antibodies

Procedure:

1. Prepare Carbonate Buffer - dilute Concentrated Carbonate Buffer 1:100 with distilled water to make 1 liter. Optional: Add 8.7g of Sodium Chloride to each liter of diluted buffer.
2. Dialyze 250 μg of the antibody to be conjugated against Carbonate Saline Buffer overnight. Ideally dialyze in buffer 100 times the antibody volume and change the buffer three times - with the first change after one hour of dialysis.
3. Remove the antibody from dialysis and bring it to 2.0 mg/ml by either concentrating or diluting.
4. Add 850 μl of Solvent Reagent to one vial of FITC and mix thoroughly. **NOTE:** FITC is unstable in direct light. Store this component in the dark and freeze for long-term storage
5. Pipette 500μl of the FITC solution prepared in Step 3 into the antibody solution from Step 3 and vortex. Mix end-over-end for two (2) hours at room temperature.
6. Dialyze the conjugate mixture from Step 5 (in the dark) against a 1:10 dilution of PBS Concentrate overnight.

Determining the Molar FITC/ Protein Ratio:

1. Read the absorbance of the FITC-antibody conjugate at λ=280nm and λ=495nm (use PBS containing 0.1% NaN_3 as the blank.
2. Calculate the molar F/P ratio using the following formula:

$$\frac{(2.77)(A_{495})}{A_{280}-(0.32\times A_{495})}$$

Estimating the Conjugate Concentration:

Calculate the estimated conjugate concentration using the following formula:

$$\frac{A_{280}-(0.32\times A_{495})}{1.4}$$

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KIT LOT NUMBER: T164G

COMPONENTS

Part #	Lot #	Description	Quantity
99 -	T126F	FITC	5 x 0.85 mg
100 -	V101D	Solvent Reagent - DMF	5.0 ml
101 -	T006A	Carbonate Buffer Concentrate	100 ml
102--	T124F	Phosphate Buffered Saline (PBS) Concentrate – 10X (Containing 0.09% NaN_3)	100 ml
190-	V102D	Sodium Chloride (Added for convenience.) Some customers, on a few occasions, have discovered that their antibodies precipitate in carbonate buffer without saline. To solve this potential problem we have included sodium chloride in bulk. To make Carbonate Buffered Saline add 8.7 grams of Sodium Chloride to each Liter of Carbonate Buffer.	100 grams

THESE PRODUCTS ARE LABORATORY REAGENTS AND ARE NOT TO BE ADMINISTERED TO HUMANS OR TO BE USED FOR ANY DRUG PURPOSE.

FOR RESEARCH USE ONLY

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