Recommended Usage of The Conjugate

The FITC-conjugate can be used directly at a dilution of 1:100-1:1000. High background in IHC/IF is usually due to the use of excessive conjugate, improper conjugate diluents or blocking of coated antigens. It may be necessary to use BSA (0.1%) or other carrier proteins in diluents to keep the background to an acceptable range.

FITC-conjugation may require fine adjustments between the ratios of FITC and conjugating protein. This will depend upon the availability of free amino groups on target protein and the level of FITC desired. It is recommended that the user try several concn. of FITC and test the conjugate in a given assay. It is therefore recommended that the user perform several mini-reactions and test conjugates.

Related Items Available from ADI

80220 HRP Conjugation kit 1 kit

80230 FITC Link (FITC-Conjugation kit 1 kit

80012 High binding ELISA Strips plates (8 wellsx12 strips) 10/pk

80050 ELISA Plate Coating buffer concentrate (10X) 50 ml

80051 Phosphate Buffered Saline (PBS, pH 7.4) (20X) 100 ml

80062 ELISA Plate Blocking Buffer , (10X) milk-based 100 ml

80070 Antibody and Conjugate Diluents for ELISA (10X), 50 ml

80080 Wash buffer concentrate (20X) for ELISA 100 ml

80091 TMB substrate (1-component) for ELISA 500 ml

80100 Stop solution for TMB substrate (ELISA) (10X), 50 ml

80150 ELISA Kit for the detection of Mouse Antibodies 1 kit

80155 ELISA Kit for the detection of Rat Antibodies 1 kit

80160 ELISA Kit for the detection of Rabbit Antibodies 1 kit

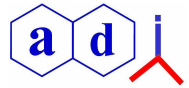
80190 Western blot Kit for mouse or rabbit, human or goat antibodies using TMB color substrate 1 kit

80200 Enhanced NuGlo Western blot kit for HRP 1 kit

Instruction Manual No. M-80230

FITC-Link Antibody/Protein conjugation to FITC

Cat. No. 80230



ALPHA DIAGNOSTIC
INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

Introduction

FITC Labeling Kit provides a simple and rapid method of labeling antibodies or other proteins with fluorescein. FITC-antibody conjugates are useful in many applications, such as flow cytometry and immunocytochemistry. Other proteins such as steroids may also be labeled with FITC. Direct labeling of the primary antibody eliminates the need for a secondary antibody, and therefore results in a lower background and an overall higher signal to noise ratio. This kit contains sufficient reagents to perform 2 indpendent conjugations of proteins. The kit contains sufficient reagents to coupel FITC up to 10-20 mg of single antibody or protein or 10-20 different proteins and antibodies.

FITC Labeling Kit requires a free amino group on the protein, antibody or peptides. The conjugating protein should be free from amine containing buffers (Tris, etc.) to improve coupling efficiency.

Kit Components:

1. FITC, 2 x ~1 mg (Protect from light. FITC and FITC conjugated proteins are unstable in direct light), Cat # 80231
2. FITC Solvent, 2.0 ml; Cat # 80232
3. Conjugation Buffer Concentrate (100X) 25 ml, #80233
4. PBS Buffer Conc. (10X; with 1% sodium azide) 25 ml, Cat # 80234
5. Dialyses tube (cut-off >3 Kda) # Cat # 80235

Reagents Required But not Provided

1. PBS, pH 7.4 for Dialyzing the conjugate (dissolve 0.26 g KH_2PO4, 2.17 g $Na_2HPO4.7H_2O$, and 8.71 g of NaCl in 1L H2O).

FITC Conjugation Protocol

1. Prepare 1x solutions of Conjugation buffer and PBS by diluting it in water.
2. Dialyze the protein or antibody in conjugation buffer overnight at 4oC in. Suggested antibody/protein cocn. is 1-2 mg/ml. If the protein is in powder form and in water or PBS then it is possible to $1/100^{th}$ v/v of the conjugation buffer (e.g. to 1 ml of protein solution in water or PBS, add 10 ul of 100x concn of the conjugation buffer). If the protein is in any other buffer then it must be dialyzed. **Note**: No Tris or other amine containing buffers be used in the protein and it must be dialyze to exchange the buffer. Dialyze in 100-200 times the solution of protein (e.g. 1 ml protein in 200 ml buffer or 0.1 ml protein in 100 ml buffer should be sufficient). Use the supplied dialyses tube after washing with ample distilled water (pre-soak the tube in water for 15 min or longer and then wash). Dialyzes tube can be washed and used again in step 6.

3. Take the dialyzed protein in an Appendorf tube or dissolve the protein in conjugation buffer at 1-2 mg/ml. If the conjugating protein is not IgG, save small aliquot for measuring absorbance before conjugation for calculating FITC/Protein molar ratio).
4. Dissolve FITC in FITC solvent (1 vial of FITC in 0.85 ml)
5. For 1 mg protein add 200 ul of FITC (for 2 mg protein add 400 ul; adjust the volume of FITC according to the protein amount). FITC solution should be added slowly, drop-wise with gentle mixing. The conjugation reaction should be done in a **brown or black** glass (preferred) or plastic vial with tight cap. If brown vials are not available then the tube should be covered with aluminum foil. Gently mix, end-over-end, for 2 hours at room temp.
6. Remove the protein and dialyze it in the supplied bag (dialyses clips are required but not provided) against 1x PBS overnight at 4oC.
7. Store FITC-conjugate protein or antibody at 4oC for Short term and frozen in small aliquots at –20oC or below.
8. FITC/Protein Molar Ratio can be calculated by measuring A280 and A495 before and after the conjugation. The detailed method is supplied with the kit.

Determining the FITC/Protein Molar Ratio:

1. Read the absorbance of the FITC-antibody conjugate at two wavelengths: 280 nm and 495 nm. Use the 1X PBS as the spectrophotometric blank. Optimal absorbance readings of the conjugated protein will be within the range of 0.2 - 1.2 OD.

2. Using the two absorbance readings, calculate the F/P molar ratio of the FITC/Protein conjugate using the following equation:

Molar F/P = Mole FITC/Moles Protein

$$\text{Molar F/P} = \frac{2.77 \times A_{495}}{A_{280} - (0.32 \times A_{495})}$$

3. There are 2 equations used for the determination of the concentration of the FITC-antibody conjugate. If the protein used for conjugation was IgG, use the following equation to estimate the concentration of the labeled antibody:

$$\text{Antibody (mg/ml)} = \frac{A_{280} - (0.32 \times A_{495})}{1.4}$$

If the protein conjugated was not IgG, use the following equation to estimate the concentration of conjugated protein:

$$\text{Protein X (mg/ml)} = \frac{A_{280} - (0.32 \times A_{495})}{E^{0.1\%}}$$

Where E0.1% is the A280 reading of a 1 mg/ml solution of the unconjugated protein, as measured in a cuvette of 1.0 cm path length.