

FITC IgG Labeling Kit

For labeling 1 x 1 mg IgG

REAGENT STORAGE

Kit Component	Storage Temp	Storage Notes
FITC Labeling Reagent	-20°C	Keep the vial in the desiccated container as supplied in the kit
Quenching Reagent B	-20°C or 2-8°C	Does not need to be kept desiccated.
100 mg/ml BSA (with 0.05% Sodium Azide)	-20°C or 2-8°C	Does not need to be kept desiccated.

INTRODUCTION

The FITC-IgG Labeling Kit utilizes a novel chemistry to generate highly reproducible fluorescein-labeled IgG with a simple procedure.

FEATURES

- Pre-measured active labeling reagent.
- Can be used with up to 10 mg/ml (1%) BSA as a carrier protein.
- Completely scalable: conjugate anywhere from 0.1 to 1 gram IgG per reaction.
- Highly efficient FITC incorporation.
- Optimized FITC:IgG ratio by labeling time and temperature.
- Purification not usually required.

PRODUCTS and CONTENTS

Components	Storage Conditions
FITC Labeling Reagent for 1 mg IgG	1 X 50 µL
Quenching Reagent B	250 µL
100 mg/ml BSA (with 0.05% Sodium Azide)	250 µL

ADDITIONAL REAGENTS REQUIRED

None

SHELF LIFE

The performance of the product is guaranteed for a minimum of 12 months when stored as directed.

IgG Amount and Concentration and Buffers

The IgG to be labeled should be at a concentration 0.5 - 2.0 mg/ml in 1X PBS, pH 7.2 – 7.5. The IgG solution may contain up to 10 mg/ml (1%) BSA.

CONJUGATION PROCEDURE for 1 mg of IgG

Note: Fluorescein is light sensitive. Perform reactions in the dark and keep light exposure to a minimum.

1. Let kit come to room temperature for at least 1 hour. Kit can be removed from -20°C up to 24 hours before use.
2. Spin the screw-cap tube containing FITC Labeling Reagent for 1 minute at high speed (> 14,000 x g).
3. Add 1 mg IgG solution to the tube containing the FITC Labeling Reagent.
4. Vortex thoroughly for 10 seconds, then shake solution down to the bottom.
5. Incubate the labeling reaction at 37°C for 60 minutes in a heat block.

Note: Room temperature incubations will be successful. 15 minute – 4 hour incubation at 37°C will be successful.

6. Remove excess FITC from the reaction by size exclusion chromatography (see Recommended Accessories section for spin desalting columns). Then proceed to step 8 – quenching is not necessary.
7. Add Quenching Reagent to the tube (not necessary if excess FITC has been removed by size exclusion chromatography):
 - For 1 mg IgG, add 100 µL per reaction.
8. Store FITC-labeled IgG in the dark at 2-8°C.

Optional: Add 100 mg/ml BSA solution to achieve the desired final concentration of BSA.

Optional: Add glycerol to a final concentration of 40-50%.

RECOMMENDED ACCESSORIES

To remove excess FITC from the labeled IgG - Order from ThermoFisher:

Sample Size	Description	Cat #
2 – 12 μ L	Zeba Spin Desalting Columns, Micro (75 μ L), 7K MWCO	89877, 89878
30 - 130 μ L	Zeba Spin Desalting Columns, 0.5mL, 7K MWCO	89882, 89883
200 – 700 μ L	Zeba Spin Desalting Columns, 2mL, 7K MWCO	89889, 89890
500 – 2000 μ L	Zeba Spin Desalting Columns, 5mL, 7K MWCO	89891, 89892
700 – 4000 μ L	Zeba Spin Desalting Columns, 10mL, 7K MWCO	89893, 89894

For concentrating IgG before labeling or for concentrating the final FITC-labeled IgG – Order from MilliporeSigma:

Sample Size	Description	Cat #
Up to 500 μ L	Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-50 membrane	Z740176
Up to 2 mL	Amicon Ultra-2 Centrifugal Filter Unit with Ultracel-50 membrane	UFC205024
Up to 4 mL	Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-50 membrane	UFC805008
Up to 15 mL	Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-50 membrane	Z648000