FITC IgG Labeling Kit

For labeling 1 x 100 µg 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.
Zeba Desalting Column with Collection Tube	2-8°C	Does not need to be kept desiccated. Do not store at freezing temperatures.

Introduction

The IgG FITC 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 & Contents

Catalogue Number	FITC-Link-AC
For Labeling:	1 x 100 ug IgG
FITC Labeling Reagent	5 µL
Quenching Reagent B	100 µL
100 mg/ml BSA (with 0.05% Sodium Azide)	100 µL
Zeba Desalting Column with Collection Tube	1 each

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 10 mg/ml (1%) BSA.

Conjugation Procedure for 0.1 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 1.5 ml screw-cap tube containing FITC Labeling Reagent for 1 minute at high speed (> 14,000 x g).
3. Add 100 µL 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. Add 10 µL of Quenching Reagent B and vortex.
7. Incubate at room temperature in dark for 5 minutes.
8. Desalt the complete reaction volume into pure 1X PBS using the included Zeba spin column. See the attached desalting protocol.
9. 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% (Not Provided).

Recommended Accessories

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

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

Zeba Spin Desalting Column Instructions

0.5 mL columns for 30 – 130 µL Samples

Notes:

- Each column can desalt a 30-130 µL sample.
- The resin slurry contains 0.05% sodium azide.
- These columns are recommended for desalting molecules > 7000 Daltons.

Storage:

- Store columns at 2-8°C.
- Columns may be stored at room temperature for several days without adverse effects.

Additional Materials Required

- Variable-speed bench-top microcentrifuge
- 1.5 mL microcentrifuge tubes

Spin Column Preparation

1. Remove column's bottom closure and loosen cap (do not remove cap).
2. Place column in the collection tube provided.
3. Centrifuge at 1500 × *g* for 1 minute to remove storage solution.
4. Remove column cap and place a mark on the side of the column where the compacted resin is slanted upward. For all subsequent steps, place column in the microcentrifuge with the mark facing outward. Improper orientation will result in reduced desalting efficiency. It is not necessary to replace the cap for subsequent steps. Discard the flow through buffer from the collection tube.
5. Add 300 µL of buffer on top of the resin bed. Centrifuge at 1500 × g for 1 minute to remove buffer. Discard the flow through buffer from the collection tube.
6. Repeat Step 5 two additional times for a total of 3 column washes.

Sample Desalting

7. Place the column in a new microcentrifuge tube and slowly apply the complete sample to the center of the compacted resin bed.
8. Do not add a stacker after application of the sample to the resin bed.
9. Centrifuge column at 1500 × g for 2 minutes to collect desalted sample. Discard desalting column after use.

Zeba Desalting Columns are a product of Thermo Fisher Scientific Inc.

Sample Size	Description	Cat #
30 - 130 µL	Zeba Spin Desalting Columns, 0.5mL, 7K MWCO	89882, 89883