

Biotin Labeling Kit

For labeling 3 x 100 µg IgG

INTRODUCTION

The Biotin Labeling kit utilizes a novel chemistry to generate highly reproducible biotinylated IgG with a simple procedure.

FEATURES

- Room temperature-stable active biotinylation reagent.
- Pre-measured active biotinylation reagent coated onto a tube surface.
- Can be used with up to 10 mg/ml (1%) BSA as a carrier protein.
- Completely scalable: conjugate anywhere from 10 µg to 1 gram IgG per reaction.
- Highly efficient Biotin incorporation.
- Purification not usually required, but desalting columns are included to remove excess biotin.

PRODUCTS and CONTENTS

Catalog Number	CNJ93102-0.3	Storage Conditions
Biotin Reagent for labeling 100 µg IgG	3 tubes	2-8°C or room temperature - in container with desiccant
Quenching Reagent B	50 µL	2-8°C
100 mg/ml BSA (with 0.05% Sodium Azide)	250 µL	2-8°C
Zeba Desalting Column with Collection Tube	3 each	2-8°C

ADDITIONAL REAGENTS REQUIRED

1X Phosphate Buffered Saline, pH 7.2 – 7.4. (1X PBS)

REAGENT STORAGE

- Store vials containing the Biotin Reagent at 2-8°C or room temperature. Keep the vials in the white plastic jar containing desiccant packets.
- Store all other reagents and components at 2-8°C.

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 biotinylated 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 BSA and up to 0.10% sodium azide.

CONJUGATION PROCEDURE for 100 µg of IgG

1. Add solution containing 100 µg of IgG to the screw-cap tube containing the Biotin Reagent.
2. Vortex thoroughly for 15 seconds, then shake or spin solution down to the bottom of the tube.
3. Incubate the reaction at room temperature for 15 minutes. Longer incubations (up to 4 hours) will also give acceptable results.
4. Optional: remove excess biotin from the reaction by size exclusion chromatography (see attached Desalting Protocol). Then proceed to step 8 – the quenching step is not necessary.
5. Add Quenching Reagent B to the reaction tube (this step is not necessary if excess biotin has been removed by size exclusion chromatography):
 - Add 10 µL Quenching Reagent B to the reaction mixture
6. Vortex gently for 5 seconds, then shake down to the bottom.
7. Incubate the reaction for 5 minutes at room temperature.
8. Optional: add 100 mg/ml BSA solution to achieve the desired final concentration of BSA.
9. Optional: Add glycerol to a final concentration of 40-50%.
10. Store biotinylated IgG at 2-8°C or -20°C.

Zeba Spin Desalting Column Instructions

0.5 mL columns for 30 – 130 μ L Samples

Notes:

- Each column can desalt a 30-130 μ L 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 $1500 \times g$ 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 μ L of buffer on top of the resin bed. Centrifuge at $1500 \times g$ 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 $1500 \times g$ 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 μ L	Zeba Spin Desalting Columns, 0.5mL, 7K MWCO	89882, 89883

RECOMMENDED ACCESSORIES

To remove unreacted biotin from the biotinylated 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 biotin labeling or for concentrating the final biotinylated 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