

Biotin Labeling Kit-NH₂

General information

Biotin Labeling Kit-NH₂ is primarily used for the preparation of biotin-labeled IgG for enzyme immunoassay (EIA). NH₂-reactive biotin, a component of this kit, has a succinimidyl group (NHS) that reacts with an amino group of IgG and other macromolecules. This kit contains all of the necessary reagents for labeling. The labeling process is simple. Add NH₂-reactive biotin to IgG solution on a filter membrane, and incubate at 37 °C for 10 min. On the average, 5 to 8 biotin molecules conjugate to each IgG molecule. Excess biotin molecules can be removed using a filtration tube included in this kit.

Description

Cat.Number:	# BG767	BG7671	BG7672	BG7673
		1kit	1kit	1kit
		3 rxn / 100µg	1 rxn/1mg	1/2 x 10/5mg

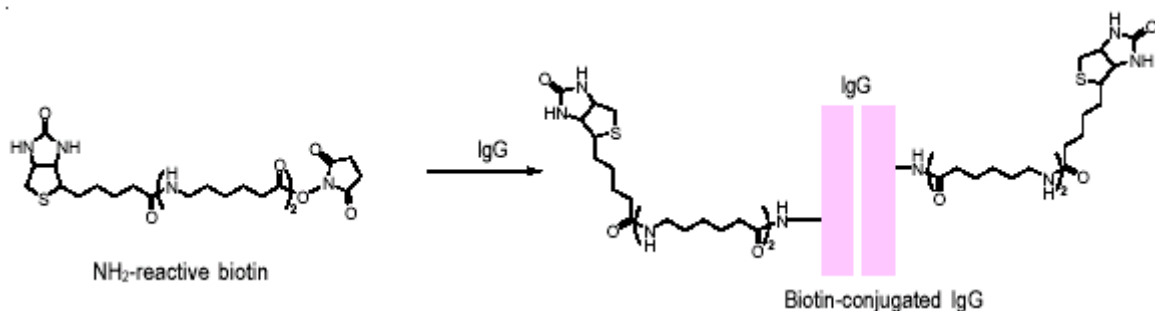
Description	Biotin labeling kit-NH₂				
	Kit contains:	- NH ₂ -reactive Biotin	100 µg x 3	1mg x 1	10mg
		- Washing buffer	4 ml x 1	10ml x 1	50ml
		- Reaction buffer	200 µl x 1	1.2ml x 1	12ml
		- Storage buffer	4 ml x 1	10ml x 1	50ml
		- Filtration tube	3 tubes	1 tube	2 tubes
				15ml centrif.tube	

Capacity: Protein (Molecular weight > 50 000, IgG: 50-200 µg)

Storage condition Store at 0-5 °C. This kit is stable for 6 months at 0-5 °C with protection from moisture.

Equipment and material non provided

- 10 µl, 200 µl and 1 ml pipettes
- Incubator (37 °C)
- Microcentrifuge



For any question,
contact your local distributor

InterBiotech,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

P.1/3

interbiotech@interchim.com, Hotline : 33(0)4 70 03 73 06

Labeling Procedure for IgG.

(for kit #BG7671/1 labeling of 10-100 μ g)



1 Add 100 μ l WS buffer and the sample solution containing 100 μ g IgG to a Filtration tube.^{a)}



2 Centrifuge at 8,000-10,000 g for 10 min.^{b)}



3 Add 10 μ l DMSO to NH₂-reactive biotin, and dissolve with pipetting.^{c)}



4 Add 100 μ l Reaction buffer to the Filtration tube, and 8 μ l NH₂-reactive biotin solution to the Filtration tube and pipette to mix.^{d)}



5 Incubate the tube at 37 °C for 10 min.



6 Add 100 μ l WS buffer to the Filtration tube, and centrifuge at 8,000-10,000 g for 10 min.^{b)} Discard the filtrate.



7 Add 200 μ l WS buffer to the Filtration tube, and centrifuge at 8,000-10,000 g for 10 min.^{b)} Repeat this step.



8 Add 200 μ l WS buffer, and pipette 10 to 15 times to recover the conjugate.^{e)} Transfer the solution to a 0.5 ml tube, and store at 0-5 °C.

a) The recommended amount of IgG is 100 μ g., but can be down 10 μ g The volume of IgG solution should be less than 100 μ l. If the antibody concentration is lower than 1 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 100 μ g. If the volume of the filtrate becomes 400 μ l or more during the accumulation process, discard the filtrate prior to go to the next centrifuge step. If the sample solution contains other proteins such as BSA, purify the antibody prior to using this kit.

Precaution: IgG or biotin-conjugated IgG is always on the filter membrane of the Filtration tube during the labeling process. If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to label biotin with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit). If the IgG solution contains small insoluble materials, centrifuge the solution, and use the supernatant for the labeling.

b) If solution still remains on the filter after the centrifugation, spin for another 5 min, or increase the centrifuge speed.

c) NH₂-reactive biotin is on the bottom of the tube. Add 10 μ l DMSO to the bottom of the tube, and pipette several times to dissolve.

d) If the amount of IgG is 200 μ g, add entire NH₂-reactive biotin solution.

e) You do not have to use WS buffer to recover biotin-conjugated IgG. You can choose any kind of buffers appropriate for your experiment.

Frequently asked questions

- Can I use this kit for other proteins or peptides?
Yes, if the molecular weight is higher than 50 000. If the molecular weight is lower than 50 000, contact our customer service at Interbiotech@interchim.com for more information.
- Do I have to use a Filtration tube prior to labeling the protein?
If the protein solution does not contain small molecules with amino groups and the concentration of the protein is 10 mg/ml or about 70 μ M, there is no need to use the filtration tube. Mix 10 μ l of the sample solution with 90 μ l of Reaction buffer, and add 8 μ l NH₂-reactive biotin solution (prepared in Step 3) to the mixture, and follow the protocol starting at Step 4.
- How long is the biotin-labeled protein stable?
If you store the biotin-labeled protein at 4°C, it is stable for 2 months. For longer storage, add 100% volume glycerol, aliquot and store at -20°C. However, please note that stability depends on the protein itself. You also can choose any kind of buffer appropriate for your experiment.
- What is the minimum amount of IgG that can be labeled using this kit?
The minimum amount is 10 μ g IgG; simply follow the protocol. The labeling ratio remains the same for 10 to 100 μ g IgG.
- How can I determine the number of biotin per protein?

For any question,
contact your local distributor

InterBiotech,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

P.2/3

interbiotech@interchim.com, Hotline : 33(0)4 70 03 73 06

The average number of biotin per IgG should be in the range of 5 and 8. If you need to determine precise number of biotin molecule per protein, use HABA assay. Following is a HABA assay protocol.

Reagent solutions:

200 μ M HABA (UP05361) solution prepared with PBS, pH 7.4	1 ml
0.5 mg avidin/ml solution prepared with PBS, pH 7.4	1 ml
diluted sample solution (55 μ l biotinylated protein solution + 110 μ l PBS, pH 7.4)	
25 μ M biotin prepared with a mixed solution (2 volumes of PBS, pH 7.4 + 1 volume of WS buffer)	200 μ l
Prepare various concentration solutions (12.5 μ M, 6.25 μ M, 3.13 μ M, 1.56 μ M) with serial dilution	200 μ l each

Assay:

1. Mix HABA solution and avidin solution in a plastic tube.
2. Add 100 μ l of the HABA-avidin solution to 15 wells for multiple assays ($n=3$).
3. Add 50 μ l biotin solution (12.5 μ M, 6.25 μ M, 3.13 μ M, and 1.56 μ M) and 50 μ l of diluted sample solution, to 3 wells each.
4. Read the O.D. at 405 nm with a reference at 492 nm, and prepare a calibration curve using the O.D. of various concentrations of biotin solutions. Read the O.D. at 280 nm to determine the protein concentration (e.g. molar absorptivity of IgG at 280 nm: 216 000).
5. Determine the concentration of biotin in the sample solution, and calculate the number of biotin molecule per protein.

Other information

For R&D use in vitro only.

Related product:

Biotining kit-SH (#BT3591)

For more information, please ask interbiotech@interchim.com

For any question,
contact your local distributor

InterBiotech,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

P.3/3

interbiotech@interchim.com, Hotline : 33(0)4 70 03 73 06