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# ab270030

# Antibody Biotinylation

# Check Kit

A product of Expedeon, an  
Abcam company

Applicable to Expedeon product codes: 4001-0030

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Antibody Biotinylation Check Kit:

[www.abcam.com/ab270030](http://www.abcam.com/ab270030)

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For the confirmation of the success of antibody biotinylation in one easy step. Suitable for use with Lightning-Link® Biotin antibody labeling kits.

This product is for research use only and is not intended for diagnostic use.

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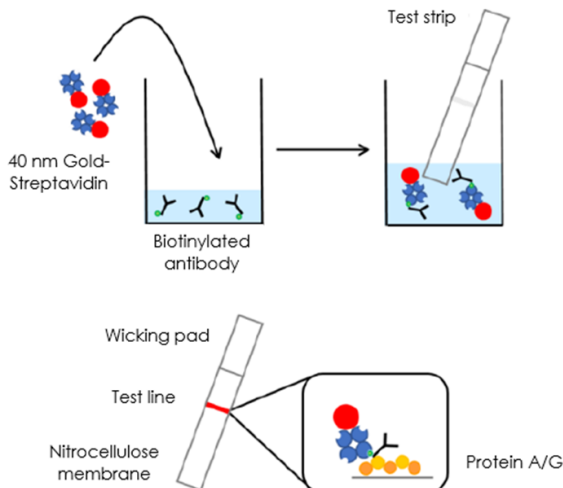
# 1. Overview

Antibody Biotinylation Check Kit (ab270030) is a quick immunochromatography test that allows you to confirm the successful conjugation of biotin to your antibody without the need for any specialized or costly equipment.

Furthermore, this qualitative lateral flow assay has the great advantage of being simpler and quicker compared to the HABA assay, traditionally used for biotinylation detection.

The key component of the kit is a nitrocellulose membrane containing a Test line of immobilized Protein A and Protein G called a half strip. Both Protein A and Protein G have a high affinity for the Fc region of a variety of IgG molecules. The half strips also contain an absorbent pad to promote and control the flow of sample through the nitrocellulose. The biotinylated antibody is run on the Protein A/G Strip together with 40 nm gold conjugated streptavidin as the detection label. Biotin binds to streptavidin with exceptionally high affinity and when the biotinylated antibody flows along the nitrocellulose, upon binding to the Protein A and Protein G concentrated on the Test line, a visible red line appears on the strip.

Antibody Biotinylation Check Kit (ab270030) is suitable for use with Lightning-Link® Biotin antibody labelling kits, as well as other biotinylation technologies.



## 2. Materials Supplied and Storage

Store Protein A/G Strips at +4°C. Store all other components at -20°C immediately on receipt. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	30 tests	Storage temperature
10X Running Buffer	2 vials	-20°C
Positive control – Biotinylated Rabbit IgG (Lyophilized)	1 vial	-20°C
40 nm Gold Streptavidin (Lyophilized)	1 vial	-20°C
Protein A/G Strips	30 Strips	+4°C

**Not supplied:** 96-wells low binding plate, Bovine serum albumin (BSA)

### 3. Technical Considerations

Protein A and Protein G affinity for immunoglobulins:

Species	Ig subclasses	Binding to Protein A	Binding to Protein G
Rabbit	IgG	High	High
Human	IgG <sub>1</sub>	High	High
	IgG <sub>2</sub>	High	High
	IgG <sub>3</sub>	No affinity	High
	IgG <sub>4</sub>	High	High
	IgA	Low	No affinity
	IgD	Low	No affinity
	IgE	Low	No affinity
Pig	IgG	High	High
	IgM	Low	No affinity
Mouse	IgG <sub>1</sub>	Low/Medium	Medium
	IgG <sub>2a</sub>	High	High
	IgG <sub>2b</sub>	High	Medium
	IgG <sub>3</sub>	Low/Medium	Medium
	IgM	Low	No affinity
Goat	IgG	Low	High
Sheep	IgG	Low	High
Rat	IgG	Low	High
	IgG <sub>1</sub>	Low	Low/Medium
	IgG <sub>2a</sub>	Low	High
	IgG <sub>2b</sub>	Low	Low/Medium
	IgG <sub>2c</sub>	Low	Low/Medium
	IgM	Low	No affinity

**Δ Note:** Low/no affinity for a specific IgG subclass may lead to low/no signal but the conjugate may be fine.

## 4. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
  
- 4.1 Dilute the 10x Running Buffer with distilled water down to 1x.
- 4.2 Reconstitute the 40 nm 10 OD Gold Streptavidin with 40  $\mu$ L of 1x Running Buffer.
- 4.3 Add 0.1% BSA (final concentration) to 1x Running Buffer
- 4.4 Dilute 40 nm Gold Streptavidin in 1x Running Buffer + 0.1% BSA 1:25 depending on the volume requested for your assay; you will need 20  $\mu$ L for each test. Remember to include the positive control.  
  
**e.g.** if you have 5 samples mix 5  $\mu$ L of 40 nm Gold Streptavidin with 120  $\mu$ L of 1x Running Buffer + 0.1% BSA.
- 4.5 Dilute your sample(s) in 1x Running Buffer + 0.1% BSA; you will need 20  $\mu$ L for each test; the optimal conjugate concentration range is 250- 15.6 ng/mL.
- 4.6 Load 20  $\mu$ L/well of your conjugate in a 96-well non-sticky plate or a suitable container.
- 4.7 Add 20  $\mu$ L of reconstituted 40 nm Gold Streptavidin 0.4 OD and mix through pipetting.
- 4.8 Insert the strip in the well.
- 4.9 Run for 10 minutes.
- 4.10 Check the result by eye. A red line will become visible if the conjugation was successful.

### Positive control – Biotinylated Rabbit IgG (Lyophilized):

- 4.11 Reconstitute the vial in 400µl of 1x Running Buffer+0.1% BSA
- 4.12 Dilute the reconstituted positive control 1:50 in 1x Running Buffer + 0.1% BSA
- 4.13 Load 20 µL/well of positive control
- 4.14 Add 20 µL of 40 nm Gold Streptavidin 0.4 OD
- 4.15 Insert the strip in the well
- 4.16 Run for 10 minutes

The positive control will produce a visible pink line indicating a successful dipstick assay.

***Δ Note:*** *The positive control was generated using Lightning-Link® Biotin Conjugation Kit Type A (ab201795).*

## Technical Support

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