

ab270051

Streptavidin Antibody Conjugation Check Kit

A product of Expedeon, an
Abcam company

Applicable to Expedeon product codes: 4004-0030

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Streptavidin Antibody Conjugation Check Kit:

www.abcam.com/ab270051

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

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

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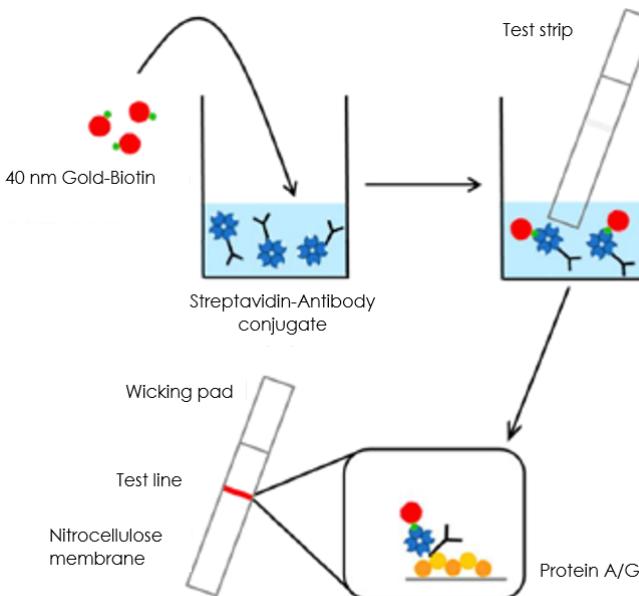
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1. Overview

Streptavidin Antibody Conjugation Check Kit (ab270051) is a quick and simple immunochromatography test that allows you to confirm the successful conjugation of streptavidin to your antibody without the need for any specialized or costly equipment.

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 streptavidin-antibody conjugate is run on the Protein A/G strip together with 40 nm gold conjugated Biotin as detection label. Biotin binds streptavidin with exceptionally high affinity and when the streptavidin-antibody conjugate flows along the nitrocellulose membrane, upon binding to the Protein A and Protein G on the T-line, a visible red line appears on the strip.

Streptavidin Antibody Conjugation Check Kit (ab270051) is suitable for use with Lightning-Link® Streptavidin antibody labelling kit.



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 – Streptavidin-Rabbit IgG Conjugate (lyophilized)	1 vial	-20°C
40 nm Gold Biotin (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 ₁	High	High
	IgG ₂	High	High
	IgG ₃	No affinity	High
	IgG ₄	High	High
	IgA	Low	No affinity
	IgD	Low	No affinity
	IgE	Low	No affinity
	IgM	Low	No affinity
Pig	IgG	High	High
Mouse	IgG ₁	Low/Medium	Medium
	IgG _{2a}	High	High
	IgG _{2b}	High	Medium
	IgG ₃	Low/Medium	Medium
	IgM	Low	No affinity
Goat	IgG	Low	High
Sheep	IgG	Low	High
Rat	IgG	Low	High
	IgG ₁	Low	Low/Medium
	IgG _{2a}	Low	High
	IgG _{2b}	Low	Low/Medium
	IgG _{2c}	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 Add 0.1% BSA (final concentration) to 1x Running Buffer

4.3 Reconstitute the 40 nm Gold Biotin with 150 μ L of 1x Running Buffer + 0.1% BSA to obtain 2 OD

4.4 Dilute your sample(s) in 1x Running Buffer + 0.1% BSA; you will need 10 μ L for each test; the optimal conjugate concentration range is 250 - 15.6 ng/mL. It is recommended to run the samples in duplicate and include the positive control (see below).

4.5 For a single test mix 10 μ L of diluted streptavidin-antibody, 4 μ L of reconstituted Gold Biotin 2 OD and 26 μ L of 1x Running Buffer + 0.1% BSA and mix through pipetting.

4.6 Load 40 μ L/well of your mix in a 96-well non-sticky plate or a suitable container.

4.7 Dip the strip in the well.

4.8 Run for 10 minutes. Check the result by eye. A red line will become visible if the conjugation was successful.

Positive control – Streptavidin-Rabbit IgG Conjugate

- 4.9 Reconstitute the vial in 300 μ L of 1x Running Buffer + 0.1% *BSA*
- 4.10 For a single run mix 10 μ L of positive control, 4 μ L of reconstituted Gold Biotin 2 OD and 26 μ L of 1x Running Buffer + 0.1% BSA and mix through pipetting.
- 4.11 Load 40 μ L/well of positive control.
- 4.12 Dip the strip in the well.
- 4.13 Run for 10 minutes.

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

Technical Support

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