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ab236555 HRP Antibody Conjugation Check Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 4002-0030.

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HRP Antibody Conjugation Check Kit:
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For the confirmation of the success of antibody conjugation to HRP in one easy step. Suitable for use with Lightning-Link® HRP antibody labeling kits.

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

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1. Overview

HRP Antibody Conjugation Check Kit (ab236555) is a quick immunochromatography test that allows to confirm the successful conjugation of Horseradish Peroxidase (HRP) to an 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 (see table "Protein A and Protein G affinity for immunoglobulins"). The half strips also contain an absorbent pad to promote and control the flow of sample through the nitrocellulose. The HRP-antibody conjugate is run on the Protein A/G Strip. The conjugate binds the Protein A and Protein G concentrated on the test line. After the addition of the HRP detection solution, a visible line appears on the strip (see Figure 1 below).

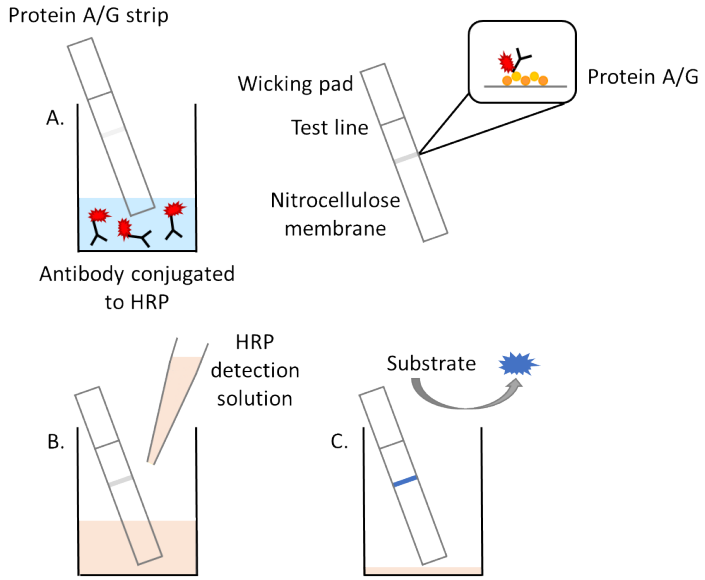

Figure 1. HRP Antibody Conjugation Check Kit (ab236555) assay procedure

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.*

2. Materials Supplied and Storage

Upon receipt, store Protein A/G Strips and the HRP detection solution at +4°C, and all other components at -20°C. 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	3 vials	-20°C
GAR-HRP (lyophilized)	1 vial	-20°C
HRP detection solution	1 vial	+4°C
Protein A/G Strips	30 Strips	+4°C

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

3. Assay Procedure

After performing the HRP-antibody conjugation reaction, the success of the conjugation can be visually checked by simply running the sample on the strips as follows:

3.1 Dilute the 10x Running Buffer with distilled water down to 1x.

3.2 Add 0.01% BSA (final concentration) to 1X Running Buffer.

3.3 Dilute the HRP-antibody conjugate in 1X Running Buffer + 0.01% BSA.

> *Δ **Note:** When using Lightning-Link® Conjugation Kits, the amount of conjugate required reflects the starting concentration of the antibody i.e. if 100 µg of antibody in 100 µL is added to a 100 µg Lightning-Link® vial, the final concentration of the conjugate is 1 mg/mL.*

> *Δ **Note:** A good range to detect the signal is 10 ng/mL to 0.5 ng/mL.*

e.g. Starting from 1 mg/mL antibody conjugate, dilute down to 1 ng/mL as follows:

- dilute 1:100: add 4 µL of 1 mg/mL conjugate to 396 µL 1x Running Buffer + 0.01% BSA to obtain 10 µg/mL concentration.
- dilute 1:100: add 4 µL of 10 µg/mL conjugate to 396 µL 1x Running Buffer + 0.01% BSA to obtain 100 ng/Ml concentration.
- dilute 1:100: add 4 µL of 100 ng/mL conjugate to 396 µL 1x Running Buffer + 0.01% BSA to obtain 1 ng/mL concentration.

3.4 Load 40 µL/well of the diluted conjugate in a 96-well non-sticky plate or a suitable container.

3.5 Insert the strip in the well with the nitrocellulose membrane dipped into the diluted conjugate.

3.6 Run for 10 minutes.

3.7 Add 40 µL/well of HRP detection solution.

3.8 Run for 15 minutes.

3.9 Check the result by eye.

The kit comes with a positive control consisting of a lyophilized GAR-HRP (generated using Lightning-Link® HRP) which should be run separately on a strip as follows:

3.10 Reconstitute the lyophilized positive control in 50µl dH2O to obtain 10 µg/mL concentration:
- Dilute 1:100: add 4µL of 10µg/mL positive control to 396 µL 1x Running Buffer + 0.01% BSA to obtain 100 ng/mL concentration.
- Dilute 1:100: add 4µL of 100ng/mL positive control to 396 µL 1x Running Buffer + 0.01% BSA to obtain 1 ng/mL concentration.

3.11 Load 40 µL/well of diluted positive control.

3.12 Insert the strip in the well.

3.13 Run for 10 minutes.

3.14 Add 40 µL/well of HRP detection solution.

3.15 Run for 15 minutes.

3.16 Check the result by eye.

*Δ **Note:** The positive control will produce a visible purple line indicating a successful dipstick assay.*

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