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ab236554
Conjugation Check Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 4000-0030.

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Conjugation Check Kit datasheet:
www.abcam.com/ab236554
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A quick immunochromatography test that allows to confirm successful conjugation of an antibody in one easy step.

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

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1. Overview

The Conjugation Check Kit (ab236554) is a quick immunochromatography test that allows to confirm successful conjugation of an antibody. 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. This simple qualitative lateral flow assay does not require any specialized or costly equipment.When the antibody conjugate is run on the Protein A/G strip, it flows along the nitrocellulose, binding to the Protein A and Protein G concentrated at the Test line. When the antibody is successfully conjugated to a colored label, a visible line appears on the strip (see Figure 1).

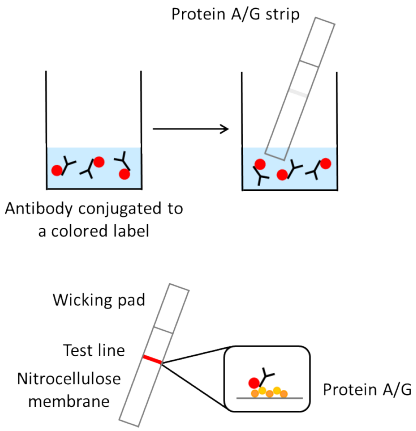

Figure 1. Conjugation Check Kit (ab236554) assay procedure

The Conjugation Check Kit (ab236554) is suitable for use with Gold Conjugation Kits, colloidal gold, and Latex Conjugation Kits, as well as most of the fluorescent Lightning-Link® antibody labeling kits. The strips can also be used with antibody conjugates prepared using other labeling technologies, provided the label is suitable for running on a lateral flow strip and has sufficient color intensity.

Δ Note: *Please note Conjugate Check Kit is not compatible with the following: AMCA, Dylight® 350, Atto390, Dylight® 405, Dylight® 800, Dylight® 755, Alex Fluor® 700 and Dylight® 680.*

Protein A and Protein G affinity for immunoglobulins:

Species	Ig subclass	Binding to Protein A	Binding to Protein G
Rabbit	IgG	High	High
Human	IgG1	High	High
	IgG2	High	High
	IgG3	No affinity	High
	IgG4	High	High
	IgA	Low	No affinity
	IgD	Low	No affinity
	IgE	Low	No affinity
	IgM	Low	No affinity
Pig	IgG	High	High
Mouse	IgG1	Low/medium	Medium
	IgG2a	High	High
	IgG2b	High	Medium
	IgG3	Low/medium	Medium
	IgM	Low	No affinity
Goat	IgG	Low	High
Sheep	IgG	Low	High
Rat	IgG	Low	High
	IgG1	Low	Low/medium
	IgG2a	Low	High
	IgG2b	Low	Low/medium
	IgG2c	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 the pot containing the strips at +4°C, and the pot containing the 10x Running Buffer and Positive Control at -20°C.

Item	Quantity	Storage temperature
Protein A/G Strips	30 units	+4°C
Conjugation Checking Kit Positive Control	1 vial	-20°C
10X Running Buffer	2 vials	-20°C

Reagents are ready to use as supplied.

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

3. Assay Procedure

After performing the conjugation reaction with Gold, colloidal gold, Latex or Lightning-Link® kits, 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 and add a blocking agent (1% BSA final concentration) to obtain 1x Running Buffer + BSA.

3.2 Dilute the conjugate in 1x Running Buffer + BSA following the instructions for each label as specified in the sections below.

3.3 Load 40 µL/well of the conjugate in duplicate in a 96-well low binding plate or a suitable container.

3.4 Insert one strip in each well.

3.5 Run for 10 minutes.

3.6 Check the conjugation by eye.

Δ Note: The kit comes with a positive control to ensure that the half strips have run correctly. The positive control vial consists of a lyophilized antibody-gold conjugate to be run separately on the strips as follows:

3.7 Reconstitute the vial in 800 µL of 1x Running Buffer + BSA.

3.8 Load 40 µL/well of positive control.

3.9 Insert the strip in the well.

3.10 Run for 10 minutes.

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

4. Checking Conjugates

Checking Gold and colloidal gold conjugates:

To determine the effective concentration of the Gold conjugates we advise measuring the Abs_{max} using a UV-vis spectrophotometer after diluting the sample to an appropriate range for your piece of equipment (e.g. if the conjugate is at 20 OD and is diluted 1:20, the Abs_{max} for a 1 cm light path is expected to be around 1 OD). A vial of our 3 x 1 µg or 10 x 1 µg Gold Conjugation Kits generate 50 µL of 20 OD conjugate.

We recommend diluting the conjugate as follows:

Gold Nanoparticle size	Abs_{max}	Suggested range (OD) of conjugate
10 nm	520 nm	2.0 – 0.1 OD
20 nm	528 nm	1.0 – 0.05 OD
40 nm	530 nm	1.0 – 0.01 OD
80 nm	550 nm	2.0 – 0.1 OD

The above concentration ranges are also relevant to conjugates made with Gold particles from other suppliers.

Checking Latex conjugates:

The concentration of latex conjugates is expressed in % solids (w/v). A vial of our 4 x 4 µg or 10 x 4 µg Latex Conjugation Kits contain 40 µL of 1% conjugate. Before running the Latex conjugates, we recommend blocking the strips as follows:

4.1 Load 80 µL of 1x Running Buffer + BSA to the strips.

4.2 Run for 1 hour.

4.3 Leave the strips to dry at room temperature for at least 1 hour, or at +37°C for 20 minutes.

4.4 Load conjugates as described in the assay procedure section 7.

Latex beads color	Suggested range (% solids) of conjugate
Black	0.02 – 0.004%
Blue	0.02 – 0.004%
Red	0.02 – 0.004%

The Protein A/G strips have been validated on our Latex Conjugation Kit, however the concentration range can also be applied to conjugates prepared from other latex bead sources.

Checking Lightning-Link® conjugates:

When using our Lightning-Link® range of Conjugation Kits, the amount of conjugate required reflects the starting concentration of the antibody. For example, 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.

We recommend diluting the conjugate as follows:

Fluorescent label	Suggested concentration of conjugated antibody
Fluorescent protein	5 – 100 µg/mL
Fluorescent dye	100 – 1000 µg/mL
Tandem dyes	50 – 1000 µg/mL
Enzyme	Not suitable

The line intensity will vary depending on the type of label used. Fluorescent dyes require a higher concentration of conjugate to produce a visible line compared to fluorescent proteins and tandem dyes. The intensity of the line will also depend on the color of the label being used.

This protocol is not suitable for enzyme conjugates.

5. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)