



Conjugate Check&Go!

Applicable to:

4000-0030 Conjugate Check&Go!

Release 1

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Introduction

Conjugate Check&Go! is a quick chromatography test that allows you to confirm successful conjugation of your 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 Appendix 1). 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 Conjugate Check&Go! strip, it flows along the nitrocellulose binding to the Protein A and Protein G concentrated on the Test line. When the antibody is successfully conjugated to a colored label, a visible line appears on the strip (see Figure 1 below).

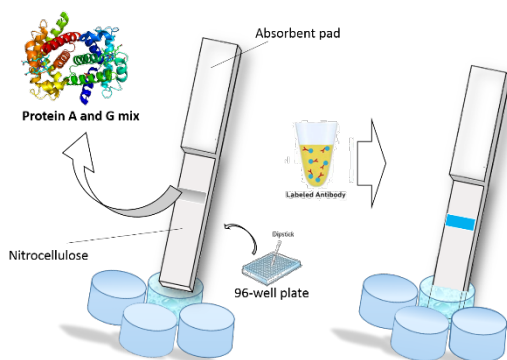


Figure 1.

Conjugate Check&Go! is suitable for use with InnovaCoat® GOLD, Colloidal Gold, and LATEX, as well as most of the fluorescent Lightning-Link® labels. 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.

Storage and shipping

The kit is shipped at ambient temperature. 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.

Kit contents

30 Conjugate Check&Go! strips

1 vial of Positive Control

1 bottle of 10x Running Buffer

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

Instructions

After performing your conjugation reaction with InnovaCoat® GOLD, Colloidal Gold, LATEX or Lightning-Link® kits, you can visually check the success of the conjugation by simply running your sample on the strips as follow:

1. Dilute the 10x Running Buffer with distilled water and add a blocking agent (1.0% BSA final concentration) to obtain 1x Running Buffer+BSA
2. Dilute your conjugate in 1x Running Buffer+BSA following the instructions for each label specified in the sections below
3. Load 40µl/well of your conjugate in duplicate in a 96-wells non-sticky plate or a suitable container
4. Insert one strip in each well
5. Run for 10 minutes
6. Check your conjugation by eye

The kit comes with a positive control to make sure 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 follow:

7. Reconstitute the vial in 800µl of 1x Running Buffer+BSA
8. Load 40µl/well of positive control
9. Insert the strip in the well
10. Run for 10 minutes
11. The positive control will produce a visible red line indicating a successful dipstick assay

Checking your InnovaCoat® GOLD and colloidal gold conjugates

To determine the effective concentration of the gold conjugates we advise to measure the Abs_{max} using an UV-vis spectrophotometer after diluting your 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 MINI vial of InnovaCoat® GOLD generates 50µl of 20 OD conjugate. We recommend to dilute the conjugate as follow:

Gold nanoparticle size	Abs _{max}	Suggested range (OD) of conjugate
10nm	520nm	2.0-0.1 OD
20nm	528nm	1.0-0.05 OD
40nm	530nm	1.0-0.01 OD
80nm	550nm	2.0-0.1 OD

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

Checking your LATEX conjugates

The concentration of LATEX conjugates is expressed in % solids (w/v). A MINI vial of Innova's LATEX Conjugation kit contains 40µl of 1% conjugate. Before running the LATEX conjugates, we recommend blocking the strips as follow:

- Load 80µl of 1x Running Buffer+BSA to the strips
- Run for 1 hour
- Leave the strips to dry at room temperature at least 1 hour or 20 minutes at +37°C
- Load your conjugate as described in the protocol

Latex bead color	Suggested range (% solids) of conjugate
Black	0.02-0.004%
Blue	0.02-0.004%
Red	0.02-0.004%

The Conjugate Check&Go! strips have been validated on Innova's LATEX Conjugation kit, however the concentration range can also be applied to conjugates prepared from other LATEX beads sources.

Compatibility with Lightning-Link® conjugates

When using Lightning-Link® the amount of conjugate required reflects the starting concentration of the antibody, i.e. if 100µg of antibody in 100µl is added to a 100µg Lightning-Link® vial, the final concentration of the conjugate is 1mg/ml.

Lightning-Link® label	Suggested Concentration of conjugated-antibody
Fluorescent protein	5-100µg/ml
Fluorescent dye	100 - 1000µg/ml
Tandem dyes	50-1000µg/ml
Enzyme	N.D.

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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. 10µg Lightning Link® kits may not be suitable for some dilutions.

This protocol is not suitable for enzyme conjugates.

Appendix

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
Pig	IgG	High	High
	IgM	Low	No affinity
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

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

Related products

Antibody labeling kits:

<https://www.innovabiosciences.com/antibody-labeling-kits.html>

Gold nanoparticles and latex:

<https://www.innovabiosciences.com/gold-nanoparticles.html>

Technical support

For further information or for any technical enquiries get in touch via our website at:

www.innovabiosciences.com/contact-us.html