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ab270537
Universal Lateral Flow Assay Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 4300-0100

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

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1. Overview

Universal Lateral Flow Assay Kit (ab270537) is designed to enable the easy development of customized sandwich lateral flow assays, by combining Lightning-Link® and Gold conjugation technologies with an immunochromatography test performed on Universal-LFA strips. The signal intensities can be qualitatively analyzed using the supplied scoring card or, for a quantitative detection, an LFA reader can be used.

The great advantage of this kit is its adaptability to any pair of capture and detection antibodies, which permits to detect of any type of analyte, without the need to strip the capture antibody onto LFA strips using expensive equipment.

The capture antibody is conjugated to Lightning-Link® Ulfa-Tag, and the detection antibody is conjugated to 40 nm Gold with our Gold Conjugation Kit, both of which require only 30 seconds to set up.

The capture and detection antibodies are diluted and incubated with the analyte and then run on Universal LFA strips. Universal LFA strips consist of a nitrocellulose membrane containing a 'Test line' (Tline) of immobilized anti-Ulfa-Tag antibody, that binds the Ulfa-Tag conjugated capture antibody which further binds the analyte in complex with the Gold-detection antibody. A red T-line appears when the analyte is present and the line intensity varies depending on the analyte concentration. Universal LFA strips also contain a 'Control-line' (C-line) that exploit the extraordinarily high affinity of streptavidin for biotin, which shows that the test is valid, and an absorbent pad to promote and control the flow of sample through the membrane.

2. Materials Supplied and Storage

Component	Amount	Storage before preparation
Lightning-Link® Ulfa-Tag conjugation kit	3 x 100 µg vials Ulfa-Tag mix 1 vial LL-Modifier 1 vial LL-Quencher	-20ºC
Gold Conjugation Kit (40 nm, 20 OD) (ab154873)	3 x cryotubes Gold 1 vial Gold reaction buffer 1 vial Gold antibody diluent 1 vial Gold quencher	-20ºC
Universal LFA strips	100 strips	+4ºC
10x Universal Running Buffer	2 cryovials	+4ºC
40 nm Gold-Biotin, 10 OD	1 vial	+4ºC
Scoring card	1	RT
96-well clear low binding plates	2	+4ºC

Not supplied: Bovine Serum Albumin (BSA)

Δ Note: *The LL-Modifier and Quencher after initial thawing can be stored at either 4°C or -20°C.*

Δ Note: *The Gold quencher, Gold reaction buffer and Gold antibody diluent can be stored at either 4°C or -20°C.*

3. Lightning-Link® Ulfa-Tag Conjugation Considerations:

The Lightning-Link® conjugation technology works by targeting amine groups (e.g. lysines) and is widely used for labellign antibodies. The conjugation protocol is optimized for labelling IgGs, although non-antibody proteins can also be labelled with this kit, provided they have free reactive amine groups. Please get in touch with Scientific Support, as protocol optimization will be needed when labelling non-antibody proteins.

3.1 Buffer Considerations:

Buffer components	
pH	6.5-8.5
Amine free buffer (e.g. MES, MOPS, HEPES, PBS)	Yes
Non-buffering salts (e.g. sodium chloride)	Yes
Chelating agents (e.g. EDTA)	Yes
Sugars	Yes
Glycerol	<50%
Thiomersal / Thimerosal	No
Merthiolate	No
Sodium Azide	<0.1%
BSA	<0.1%
Gelatin	<0.1%
Tris	<50 mM
Glycine	No
Proclin	No
Borate buffer	Yes
Nucleophilic components (Primary amines	No
e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	

Δ Note: *Individually the concentrations shown should not affect the reaction. However, in combination with additional compounds that are not recommended above a certain concentration, the reaction may be affected*

Δ Note: *If the antibody is not in a suitable buffer for conjugation, please consult our Antibody Purification Kits.*

3.2 Recommended Starting Amount and Volume of Antibody:

Vial size	Maximum Amount of Antibody	Fixed Volume of Antibody
100 µg	100 µg	100 µL

Superior conjugates are normally generated using 100 µg of antibody at 1 mg/mL. Antibodies less than 1 mg/mL can still be used to generate good conjugates provided the maximum conjugation volume is not exceeded. Please note that adding less than the maximum amount of antibody may result in unbound label post conjugation.

Antibodies below 0.5 mg/mL should be concentrated prior to use. Our Antibody Concentration and Clean-Up Kit is suitable for this purpose (ab102778).

3.3 Storage of Lightning-Link® Ulfa-Tag Conjugates:

Your Ulfa-Tag conjugate can be stored at 4ºC for up to 18 months. For longer storage, the conjugate can be stored at 20°C with a cryoprotectant such as 50% glycerol. The best storage conditions for any particular conjugate must be determined by experimentation.

4. Gold Conjugation Considerations:

The Gold Conjugation Kit allows antibodies or proteins to be covalently attached to ultra-stable Gold Nanoparticles at very high OD quickly and easily. The Gold Nanoparticles in this kit have a protective surface coat that can withstand the most extreme conditions (e.g. 2.5M NaOH at 70°C for >1 hour).

The Gold nanoparticles in this kit are freeze dried. The conjugation reaction is initiated simply by reconstituting the dry mixture with your antibody, which becomes attached (via lysine residues) to the proprietary coating on the Gold nanoparticles.

The hands-on time for the Gold conjugation procedure is about 2 minutes and the conjugate is ready to use within 20 minutes. The researcher simply pipettes the biomolecule into a vial containing the Gold nanoparticles.

4.1 Buffer Considerations:

Buffer components	
pH	6.5-8.5
Amine free buffer (e.g. MES, MOPS, HEPES)	Yes
Sugars	Yes
PBS	No
Glycerol	<50%
Thiomersal / Thimerosal	No
Merthiolate	No
Sodium Azide	No
BSA	No
Gelatin	No
Tris	No
Glycine	No
Carboxylic acids (e.g. EDTA, Citrate)	No
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	No

Δ Note: *Relatively weak buffers (e.g. 10 mM) are strongly preferred so that the pH conditions of the covalent reaction are not significantly altered upon addition of the antibody.*

Δ Note: *If the antibody is not in a suitable buffer for conjugation, please consult our Antibody Purification Kits.*

4.2 Measure Conjugate Concentration:

The maximum absorbance (Absmax) for 40 nm Gold is 530 nm. To determine the effective concentration of the conjugate obtained we advise to measure the Absmax using a 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 Absmax for a 1 cm light path is expected to be around 1 AU).

4.3 Storage of Gold Conjugates:

For any new conjugate, initial storage at 4ºC is recommended. The quencher added at the end is a good conjugate storage buffer. Do not store the conjugate at 20ºC. The bond between the nanoparticle and antibody is covalent, which means that the conjugates are very stable. The determining factor for conjugate stability will be the antibody itself, as it will be first to degrade. Therefore, as long as your antibody is stable, the conjugate will be stable as well.

5. Assay Procedure

- Note **step 5.1 takes a minimum 3.5 hours** to conjugate, plan your time.

5.1 Conjugate the capture and the detection antibodies using the Lightning-Link® Ulfa-Tag kit and Gold Conjugation Kit (40 nm, 20 OD) respectively, following the instructions below: -

Allow all of the reagents to warm to room temperature.

Lightning-Link® Ulfa-Tag Conjugation:

1. Before you add antibody to the Ulfa-Tag mix, add 1 µL of LL- Modifier reagent for each 10 µL of antibody to be labeled. Mix gently.

2. Remove the screw cap from the vial of Ulfa-Tag mix and pipette the antibody sample (with added LL-Modifier) directly onto the lyophilized material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.

3. Place the cap back on the vial and leave the vial standing for 3 hours at room temperature (20-25ºC). Alternatively, and sometimes more conveniently, conjugations can be set up and left at room temperature overnight, as the longer incubation time has no negative effect on the conjugate.

4. After incubating for 3 hours (or more), add 1 µL of LLQuencher reagent for every 10 µL of antibody used. The conjugate can be used after 30 minutes. No separation steps are necessary.

Gold Conjugation:

1. Dilute your stock detection antibody with the Gold antibody diluent provided in the kit to 0.1 mg/mL recommended concentrations as below:

   Δ Note: *If you wish to examine the effect of varying the amount of antibody, make additional stocks but do not change the volume of antibody added (see table below). In order to vary the amount of antibody added, you must change the concentration of the stock antibody and use a fixed volume.*

2. In a microfuge tube add the Gold reaction buffer and your now diluted antibody according to the table below and mix gently:

	Volume
Gold reaction buffer	42 µL
Diluted antibody	12 µL

Δ Note: *You will have more mixture than you will actually use in the conjugation reaction.*

3. Add 45 µL of reaction buffer/antibody mixture to a Mini vial, and reconstitute the Gold by gently pipetting up and down. Leave the reaction for 15 minutes at room temperature.

4. After 15 minutes, add 5 µL Gold quencher to stop the reaction, and mix well, but gently.

5. Leave the reaction for 5 minutes. You now have 20 OD conjugate (50 µL). Dilute further as required for your application.

   Optional:

6. For a conjugate 100% free from unbound antibody we recommend to wash the particles by adding 10 times the volume of the quencher diluted 1:10 in water to the conjugate (i.e. 1 mL 1:10 diluted quencher to 100 µL of conjugate) and then centrifuge it in a microfuge at 9,000 x *g* for 10 minutes

7. Carefully remove the supernatant, gently tap the pellet and add the quencher diluted 1:10 in water for long term storage in the fridge (up to 1 year) or 1:10 diluted quencher with addition of 0.5 - 2% BSA for LFA or your preferred buffer. It is important to avoid substances that have a very high affinity for Gold (e.g. thiols).

5.2 Dilute the 10x Universal Running Buffer 1:10 with distilled water and add a blocking agent (0.1% BSA final concentration) to obtain 1x Universal Running Buffer + BSA.

5.3 Dilute the Ulfa-Tag conjugated capture antibody to 40 to 150 µg/mL in 1x Universal Running Buffer + BSA.

5.4 Dilute the Gold-detection antibody to 6 OD in 1x Universal Running Buffer + BSA.

5.5 Dilute the 40 nm Gold-Biotin 10 OD to 1 OD in 1x Universal Running Buffer + BSA (1:10 dilution).

5.6 Dilute your analyte in 1x Universal Running Buffer + BSA.

5.7 We recommend testing each sample in duplicate or triplicate. For a single strip, prepare the following mix:

- 5 µL diluted capture Ulfa-Tag-conjugated antibody
- 5 µL 6 OD 40 nm Gold-detection antibody
- 5 µL 1 OD 40 nm Gold-Biotin
- 75 µL analyte solution

5.8 Incubate for 5 minutes.

5.9 In a 96 well plate, load 80 µL of the mix and insert one Universal LFA strip in each well. Handle the strip from the wicking pad (thicker pad, made of cellulose), avoid touching the nitrocellulose, and make sure the sample pad (thinner and longer pad, made of glass fiber) is dipped into the well.

5.10 Run the mix for 20 minutes.

5.11 Compare the T-line color intensity with the score reported on the scoring card. If the C-line is not visible the test is not valid and should be repeated.

6. Lateral Flow Assay Optimization

We recommend testing different concentrations of Ulfa-Tag conjugated capture antibody between 40 and 150 µg/mL, loading a fixed concentration of Gold conjugate for initial testing. Too much capture antibody will result in a low signal, as free antibody may compete with immunocomplexes for binding to the T-line.

Increasing the OD of the Gold-conjugates will increase the intensity of the T and C-lines but also the background. If your

sample contains biotin, it may cause a reduction in C line intensity: if it is not clear whether the test is valid, either dilute your sample in Running Buffer or increase the amount of 40 nm Gold-Biotin conjugate.

When running an LFA for the first time, we recommend trying large dilutions of sample/antigen to determine the dynamic range of the assay. The sensitivity of the test will vary based on the concentration and nature of the antigen to be detected. We advise to keep the volume loaded on the strips at 80 µL. The 2 x 96-wells plates provided allows you to have wells in surplus to plan with ease your experiments.

7. FAQs

1. Can I use the kit for a competitive assay?

The Universal Lateral Flow Assay kit is not suitable for competitive assays or LFA based on nucleic acids.

2. What should I do if my sample contains biotin?

If your sample contains biotin the signal intensity on the T line won't be affected; however, the control line may be less intense: we suggest loading more concentrated Gold-Biotin (keep the volumes the same) or, if possible, diluting your sample.

3. How can I check that my antibody conjugation reactions are successful?

There is no simple way to test your Ulfa-Tag antibody conjugate, you'll have to test in your assay varying the concentration suggested in the protocol; you can test your 40 nm Gold conjugate on our Conjugation Checking Kit (ab236554).

4. Do I need a wash or desalt step?

One of the advantages of the Lighting- Link® technology compared to traditional labelling methods is that conjugate purification is not required. The Lightning-Link® reactions are highly efficient, and the kits have been optimized so that, provided the protocols are followed, there should be only a very low amount of free label left at the end of the conjugation.

We recommend washing the particles adding 10 times the volume of the Gold quencher diluted 1:10 in water to the conjugate (i.e. 1 mL 1:10 diluted quencher to 100 µL of conjugate) and then centrifuge it in a microfuge at: 9,000 x *g* 10 minutes (40,60 and 80 nm gold conjugates)

5. How do I measure the particles that are bound to the T line and C line?

This depends on what you want to achieve from the lateral flow assay, and the resources that you have available. A qualitative readout is cost-effective, requiring only a visual assessment, and for this we have provided a score card. A quantitative readout is more expensive, requiring the use of an LFA strip reader. There are plenty of these devices on the market, with the price reflecting the level of sophistication. An LFA strip reader will allow you to create a calibration curve, and will provide reproducible and accurate measurements.

6. What type of linkage to gold is formed?

The antibody becomes covalently and irreversibly attached via lysine residues to the proprietary coating of the 40 nm Gold particles.

Technical Support

For all technical or commercial enquiries please go to:

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