Innova Biosciences
making science easier

FlexLISA® Kit

Applicable to:

4200-0010: FlexLISA HRP 1 x 96 well clear stripwell plate
4200-0020: FlexLISA AP 1 x 96 well clear stripwell plate
4200-0030: FlexLISA HRP 1 x 96 well black stripwell plate
4200-0040: FlexLISA AP 1 x 96 well black stripwell plate

Release 1 03/05/2017

Introduction

The Innova Biosciences FlexLISA® kits are designed for the development and optimization of sandwich ELISA assays to detect the presence of any antigen with a high affinity for any antibody pair in complex samples (serum, plasma, urine etc.). At the core of the kit, there is the 30 seconds hands-on time Lightning-Link® conjugation technology for the labeling of the capture and detection antibodies of choice and a biotin pre-coated 96 well stripwell plate provided either clear or black, depending on the enzymatic substrate. The main advantage of this format is that the amount of capture antibody is reduced up to 40-fold, allowing ELISAs to be developed in a cost-effective way using small amounts of commercially available antibodies. The format also allows more than one capture reagent to be used, allowing multiple analytes to be assessed on a single plate.

Each FlexLISA® kit allows you to conjugate, in 30 seconds hands-on time, up to 3 different capture antibodies to streptavidin using the 3 x 10ug Lightning-Link®-Streptavidin kit, and up to 3 different detection antibodies to either horseradish peroxidase (HRP) or alkaline phosphatase (AP) using the 3 x 10ug Lightning-Link®-HRP or Lightning-Link®-AP kits. A rapid one-step incubation protocol is used in this kit, employing pre-blocked microtiter well strips coated with biotin.

The FlexLISA® assay is run by adding the sample and the antibody mix to the wells, incubating for 1 hour, washing and reading the assay plate.

FlexLISA® is ideal for quick and reliable ELISA assay development, antibody pair screening and ELISA assay optimization.

Kit contents

3 x 10ug Lightning-Link® Streptavidin

3 x 10ug Lightning-Link® Horseradish Peroxidase (HRP) or Alkaline Phosphatase (AP)

1 x black or clear biotin pre-coated 96 stripwell plate (12 x 8 well strips) in a re-sealable foil pouch

Not provided: antibodies, assay buffers, enzyme detection solutions

Additional materials required

Microplate reader capable of detecting fluorescence/absorbance (depending on the enzyme substrate)

Plate washing capabilities: an automated plate washer or multi-channel pipette

Microcentrifuge tubes for standard and sample dilutions

Orbital microtiter plate shaker for incubation

Foil or other plate lid to cover plate during the incubation

Data analysis and graphing capabilities

Shipping conditions

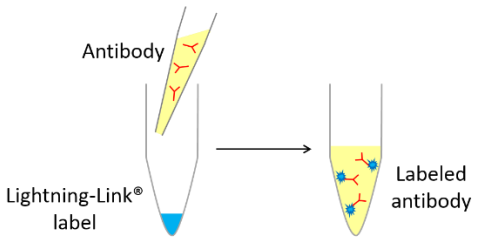
The kit is shipped at ambient temperature

Store the kit at -20°C upon receipt

All the buffers can be stored at either +4°C or -20°C

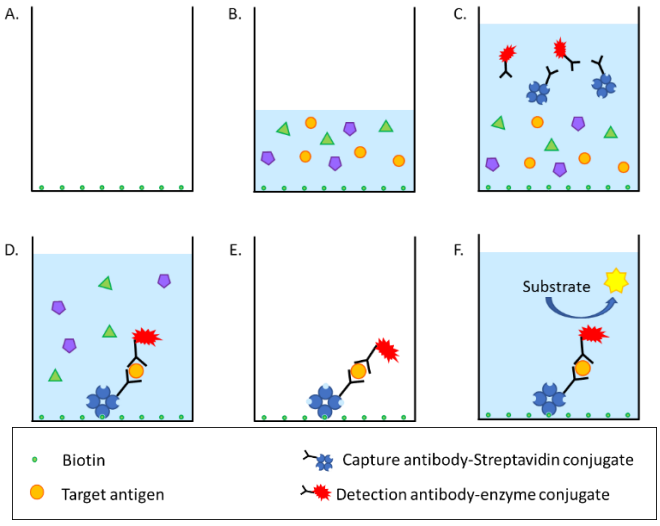
Antibody conjugation instructions

1. Allow the tamper-evident polypropylene containers to warm to room temperature.
2. Refer to the conjugation protocols provided in each Lightning-Link® kit to get 11ul 1mg/ml of streptavidin capture antibody and either HRP or AP detection antibody conjugates.



3. After quenching the reaction, store the conjugate in the fridge.

Assay principle



A. Equilibrate the stripwell plate to room temperature then prepare buffers, standards (if present), samples and the Antibody Mix

B. Add 50µl sample or standard to the well

C. Add 50µl Antibody Mix to the well
D. Incubate for 1 hour at room temperature and 400rpm
E. Wash 3 x 200µl with 1x Wash Buffer
F. Add the substrate and read the plate

Sample and reagent preparation

The necessary sample dilution is dependent on the antigen concentration in the sample. For initial testing, we recommend preparing serial dilutions of sample alongside a standard curve to determine the dynamic range of the assay.

All wash buffers and assay buffers optimized for ELISA are compatible with the kit. We recommend using a PBS/TBS buffer with the addition of a 0.01-0.1% detergent (Tween20) and, for assay buffer only, 0.1-1% blocking agent (BSA).

The Antibody Mix consists of capture antibody conjugated to streptavidin and detection antibody conjugated to either HRP or AP diluted in assay buffer. We advise all standards and samples be run at least in duplicate.

Please consider your sample may contain biotin. If so, it could interfere with the assay at concentrations > 200pg/ml. In most cases dilutions of the sample will reduce and neutralize the free biotin in the assay, but if you are concerned it may interfere, perform a pre-incubation (30-60min) with just the Antibody Mix and then add the sample.

Prepare the volume required of Antibody Mix in assay buffer in slight excess referring to the table below (e.g. for 12 stripwells we recommend making 6ml of Antibody Mix):

	1mg/ml stock capture Ab-streptavidin	**1mg/ml stock detection Ab - HRP**	**1mg/ml stock detection Ab - AP**
Antibody Mix concentration	**0.5ug/ml**	**0.5ug/ml**	**1.7ug/ml**
for 6ml of Antibody Mix	*3ul*	*3ul*	*10ul*

Assay protocol

1. Equilibrate the stripwell plate to room temperature and prepare the samples and reagents as described above.
2. Add the required number of stripwells to the plate frame and return any remaining strips to the foil pouch and seal it.
3. Add 50µl of each sample or standard to each well.
4. Add 50µl of the Antibody Mix to each well.
5. Incubate the plate on a plate shaker at room temperature and 400rpm for 1 hour.
6. Discard the solution and add 200µl wash buffer to each well using a multi-channel pipette or plate washer. It is important to remove as much liquid as possible. Repeat this two more times (i.e. three wash steps in total) and gently tap plate dry on absorbent paper towels.
7. Add 100ul of the substrate solution, incubate and detect the signal using a microplate reader (absorbance/fluorescence are substrate specific).

The table below contains the most common substrates for HRP and AP:

HRP	AP
TMB, QuantaRed™ QuantaBlu™, AmpliFlu™ Red	PNPP, 4-MUP

Troubleshooting

Problem	**Possible Cause**	**Recommended Solution**
High background	Insufficient washing	Increase the number of wash steps
	Insufficient sample dilution	Increase sample dilution
	Contaminated detection solution	Repeat assay
Variable Replicates	Poor pipetting technique	Check against best practice. We advise reverse pipetting for standard, sample and Antibody Mix loading
	Contamination of samples during addition to plate	Check against best practice
	Blocked plate washer	Check all plate washer channels
Low Signal	Insufficient assay incubation time	Increase assay incubation time to 2 - 5 hours
	Insufficient substrate solution incubation time	Check substrate solution detection protocol (supplier)
	Incorrect plate reader settings	Check settings used

Related products

Lightning-Link® Streptavidin:
https://www.innovabiosciences.com/antibody-labeling-kits/biotin-streptavidin/lightning-link-streptavidin.html
Lightning-Link® HRP:
https://www.innovabiosciences.com/antibody-labeling-kits/enzymes/immunohistochemistry-lightning-link-horseradish-peroxidase-hrp.html
Lightning-Link® AP:
https://www.innovabiosciences.com/antibody-labeling-kits/enzymes/lightning-link-alkaline-phosphatase-ap.html

Technical support

For further information or for any technical enquiries get in touch via our website at:
www.innovabiosciences.com/contact-us.html

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