

# CaptSure™ DIY ELISA

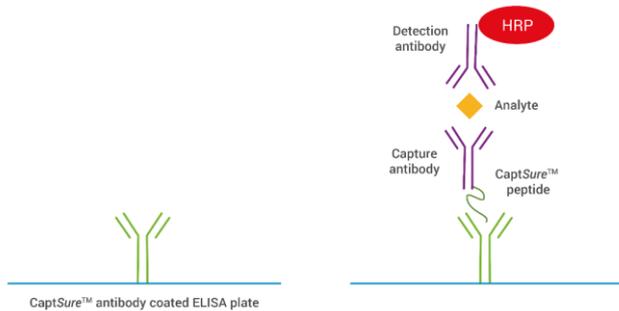
Applicable to 6300020

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## INTRODUCTION

CaptSure DIY ELISA is the latest cutting-edge ELISA kit with a built-in system for antibody labeling, enabling the rapid and easy development of in-house ELISAs using any antibody pair, while eliminating the need to perform plate coating procedures.

CaptSure DIY ELISA is powered by the combination of Expeleon's proprietary CaptSure and Lightning-Link® technologies, enabling you to reduce the amount of capture antibody required, measure different targets on the same plate with different assay antibody pairs, and streamline your ELISA to a single-wash format if desired.



Schematic representation of CaptSure technology.

The CaptSure DIY ELISA kit has a ready-to-go CaptSure assay plate, pre-coated with our CaptSure antibody, which is specific for the CaptSure peptide. The capture antibody is quickly and easily conjugated with the Lightning-Link CaptSure peptide supplied within the kit. Once added to the plate, the interaction between the CaptSure antibody and the CaptSure peptide immobilizes the immune-complex to the plate.

The detection antibody is similarly conjugated with HRP using the Lightning-Link HRP conjugation reagent, also supplied in the kit. Thus, with minimal hands-on time, you can set up both of your antibodies to ensure the best performance of your ELISA.

## KIT CONTENTS

### CaptSure DIY ELISA Buffer Pack

- 1x HRP-Labelled Antibody Dilution Buffer
- 1x CaptSure Peptide tagged Antibody Dilution Buffer
- 1x Detection Reagent
- 1x Stop Solution
- 1x Wash Buffer
- 2x Sample Dilution Buffer (Secreted Proteins)
- 1x Lysis Extraction Buffer (5X) (Cellular Proteins)

### CaptSure DIY ELISA Conjugation Pack

- 5x CaptSure Assay Plates
- 1x Lightning-Link CaptSure Peptide Conjugation Kit
- 1x Lightning-Link HRP Conjugation Kit

## SHIPPING & STORAGE CONDITIONS

The kit is shipped at +4°C. Store the buffer pack at +4°C. CaptSure Assay Plates can be stored at either +4°C or room temperature. If stored at +4°C, the strip wells should be allowed to equilibrate to room temperature prior to opening the pouch to minimize condensation. If less than a whole plate is used in an assay, place the unused strips back in the foil pouch, seal and store with desiccant at +4°C for up to 1 month.

Store both the Lightning-Link CaptSure Peptide Conjugation Kit and the Lightning-Link HRP Conjugation Kit at -20°C upon receipt.

Please note that the modifier and quencher after initial thawing can be stored at either +4°C or -20°C.

## REAGENT & BUFFER PREPARATION

To ensure a successful outcome of your assay, it is crucial all reagents and buffers are prepared according to the instructions below. Please read them carefully before starting the assay.

### Capture and detection antibody preparation

- Conjugate the capture and detection antibodies to Lightning-Link CaptSure Peptide and Lightning-Link HRP, respectively, following the corresponding labeling protocols provided separately;
- To ensure optimal performance, we recommend to conjugate Lightning-Link CaptSure Peptide to the capture antibody at 1mg/ml;
- To ensure optimal performance, we recommend to conjugate Lightning-Link HRP to the detection antibody at 2 mg/ml;
- The capture antibody conjugated to Lightning-Link CaptSure peptide should be diluted in the CaptSure Peptide tagged Antibody Dilution Buffer just prior to assaying and needs to be 4x the desired final concentration;
- The detection antibody labeled with Lightning-Link HRP should be diluted in the HRP-Labelled Antibody Dilution Buffer just prior to assaying and needs to be 4x the desired final concentration;
- The optimal working concentrations of both CaptSure Peptide tagged Antibody and HRP-Labelled Antibody should be determined by titration. The final concentration in the well is typically 100-500 ng/ml and 50-250 ng/mL in 100µl for CaptSure Peptide tagged Antibody and HRP-Labelled Antibody, respectively;

### Antibody Mix Preparation

- To adequately cover a full 96-well plate, 3mL of each CaptSure Peptide tagged Antibody and HRP-Labelled Antibody are required. If using less than a full CaptSure Assay plate (96-well), adjust the volumes accordingly;
- Mix the CaptSure Peptide tagged Antibody and the HRP-Labelled Antibody 1:1 to generate an Antibody Mix. To cover a full CaptSure Assay plate (96-well), 6ml of Antibody Mix are needed;

### Sample preparation

- When diluting the sample, select the appropriate buffer according to the type of sample being used. Sample Dilution Buffer is optimized for secreted and recombinant proteins, while Lysis Extraction Buffer (5X) is recommended for cellular proteins and cell lysates;

- Prepare 1X Lysis Extraction Buffer by mixing H<sub>2</sub>O and Lysis Extraction Buffer (5X) (e.g. 4mL H<sub>2</sub>O + 1mL of Lysis Extraction Buffer (5X))
- Prepare 1X Wash Buffer by mixing H<sub>2</sub>O and Wash Buffer (10X) in a 9:1 ratio (e.g. 9mL H<sub>2</sub>O + 1mL Wash Buffer (10X)). For a single 8-well strip, ~5mL 1X Wash Buffer is needed;

## INSTRUCTIONS

The following protocols require the preparation of cellular lysates in a separate 96 well tissue culture microplate. The lysate is subsequently transferred to a CaptSure assay plate for the assay.

### Lysate preparation – Adherent cells

1. Remove any media and cellular treatments from the cells
2. Optional: Wash cells with PBS
3. Lyse the cells with 100µL/well of 1X Lysis Extraction Buffer, under shaking (~300rpm) at room temperature for 10 minutes. The 1X Lysis Extraction Buffer volume should be adjusted depending on the desired lysate concentration. The recommended lysate concentration is 0.1–0.5 mg/mL. However, preparing more concentrated lysates can help with the detection of low abundance analytes.

### Lysate preparation – Nonadherent cells

1. Plate the cells at an appropriate density in culture medium. A cell density that yields cellular lysate at a protein concentration of 0.1–0.5 mg/mL is suitable for many cell lines
2. Optional: Return the cells to a 37°C incubator for 1–2 hours. For certain pathways, this can allow handling mediated pathway activation to subside. This step depends on the activation status of the cells following resuspension
3. When cell treatment is completed, lyse the cells with 1/5 final volume of Lysis Extraction Buffer (5X), with shaking (~300rpm) at room temperature for ten minutes (e.g. for 40 µL of cells, use 10 µL of Lysis Extraction Buffer (5X)).

### ELISA protocol

1. Equilibrate the CaptSure assay plate sealed in foil pouch to room temperature. Remove enough strips for each experiment. Return unused strips from the frame and reseal in the storage pouch and place at +4°C.
2. Add 50 µL/well of sample to the CaptSure assay plate
3. Optional: Add 50 µL/well of Sample Dilution Buffer or 1X Lysis Extraction Buffer to separate wells to assess the background signal
4. Add 50 µL/well of Antibody Mix to the CaptSure Assay Plate
5. Cover the microplate with the adhesive plate seal and incubate for 60 minutes at room temperature on a microplate shaker (~300rpm)
6. Wash wells with 200 µL/well of 1X Wash Buffer
7. Repeat the previous washing step two additional times. If using a microplate washer, use 3x washes with a 10-second mixing time between each wash
8. Remove any remaining wash buffer from wells by tapping the plate onto dry tissue and tapping gently.
9. Add 100 µL/well of Detection Reagent. The Detection Reagent should be added immediately after plate washing. Do not allow the microplate to dry. If necessary, leave the microplate in 1X Wash Buffer for up to 30 minutes, until ready to add Detection Reagent
10. Cover the microplate with an adhesive seal and foil and incubate for 15 minutes at room temperature on a microplate shaker (~300rpm)
11. Add 100 µL/well of Stop Solution and mix briefly (5–10 secs) on a microplate shake (~300rpm)
12. Read absorbance at 450 nm

## STORAGE OF CONJUGATES

Once conjugated, store the CaptSure Peptide tagged antibody and the HRP-labeled antibody in 50% glycerol at -20°C for up to 2 years (100–200 µg/mL). However, the best storage conditions for any particular conjugate must be determined by experimentation.

Our LifeXtend™ HRP conjugate stabilizer/diluent (product code 901-0005) is a proprietary multi-component reagent system that protects antibody-HRP conjugates thus ensuring the best possible performance in experiments performed at room temperature.

## TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: [technical.enquiries@expedeon.com](mailto:technical.enquiries@expedeon.com)

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