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ab270690 SimpleStep ELISA® CaptSure™ Peptide Conjugation Kit

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

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1. Overview

SimpleStep ELISA® CaptSure™ Peptide Conjugation Kit (ab270690) allows the CaptSure™ Peptide conjugation to be set up in less than 30 seconds, simply by adding a solution of the antibody to a lyophilized mixture containing a proprietary activated peptide.

By circumventing the desalting or dialysis steps that commonly interrupt traditional antibody conjugation procedures, this process can be used to label both small (e.g. 20 µg) and large quantities of primary antibodies with ease. Batch-to-batch variation upon scale-up is minimal as the process is so simple, and recoveries are always 100%.

It can also be used to label proteins, peptides and other biomolecules.

Capture antibodies tagged with CaptSure™ Peptide are paired with detection antibodies labeled with HRP to be then used within SimpleStep ELISA® Custom ELISA Kit (ab270552). Upon addition to the Assay Plate, the non-biological CaptSure™ Peptide is bound with high affinity to the antibody, pre-coated on the plates, and the immuno-complex is thus immobilized into the well.

This technology works by targeting free amine groups. It can be used to label antibodies, peptides, proteins and other molecules with free amine groups.

The protocol provided here is optimized for labeling IgGs.

2. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

3. Storage and Stability

- The kit is shipped at +4°C.
- Store the 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.

4. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

5. Materials Supplied

Item	Quantity	Storage Condition
CaptSure™ Peptide	3 vials	-20°C
CaptSure™ Modifier Reagent	1 vial	+4°C/-20°C
CaptSure™ Quencher Reagent	1 vial	+4°C/-20°C

6. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

7. Buffer Considerations

Buffer Components	Compatible
Purified antibody	Yes
Antibody in ascites fluid, serum, hybridoma or tissue culture media	No
pH	6.5-8.5
Amine free buffers (e.g. MOPS, MES, HEPES, PBS)	Yes
Non-buffering salts (e.g. NaCl)	Yes
Chelating agents (e.g. EDTA)	Yes
Sugars	Yes
Glycerol	≤ 25%
Thiomersal/ Thimerosal	No
Merthiolate	No
Sodium Azide	≤ 0.25%
BSA	No
Gelatin	No
Tris	≤ 50 mM
Glycine	No
ProClin 300	≤ 0.01%
Borate buffer	Yes
Nucleophilic components (e.g. amino acids, ethanolamine, mercaptoethanol or DTT)	No

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 requires being concentrated or cleaned-up, we recommend using the Antibody Concentration And Clean-Up Kit (ab102778).

8. Amount and Volume of Antibody

- This process has been optimized to enable the flexible labeling of 20 μg to 200 μg of antibody. High performing antibody conjugates can be generated over a broad antibody concentration range of 0.2 - 2.0 mg/mL in a 100 μL reaction volume. We recommend not altering the reaction volume as this will alter the kinetics of the conjugation reaction.
- If antibodies are used at the lower concentration (e.g. 0.2 mg/mL), excess free CaptSure™ Peptide will be present post conjugation. This excess label will be deactivated by the quencher and does not pose a problem when used on the pre-coated assay plates. Surplus CaptSure™ Peptide binding sites are available on the pre-coated assay plates to accommodate excess free label.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.

- 9.1 To your 100 μL antibody solution, add 10 μL of CaptSure™ Modifier Reagent and mix gently.
- 9.2 Remove the screw cap from the vial of CaptSure™ Peptide and pipette the antibody sample (with added CaptSure™ Modifier Reagent) directly onto the lyophilized material.
- 9.3 Resuspend the lyophilized material by gently pipetting up and down.
- 9.4 Place the cap back on the vial and leave the vial standing for 45 mins at room temperature (20-25°C).
- 9.5 After incubating for 45 mins, add 10 μL of CaptSure™ Quencher Reagent and incubate for 15 mins. No separation steps are necessary.

10. Storage of Conjugates

- CaptSure™ Peptide-tagged antibodies can be stored at -20°C with a cryoprotectant such as 50% glycerol. Under such conditions, antibody conjugates are generally stable for ≥ 2 years.
- The best storage conditions for any particular conjugate must be determined by experimentation.

11. Notes

Technical Support

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