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ab270552 SimpleStep ELISA® Custom ELISA Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 6300020

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

Table of Contents

1. Overview

SimpleStep ELISA® Custom ELISA Kit (ab270552) 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.

It is powered by the combination of proprietary 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.

The capture antibody of interest is quickly and easily conjugated with the Capture Peptide supplied within the kit. The interaction between the antibody coated on the plate and the Capture Peptide immobilizes the immune-complex.

The detection antibody is similarly conjugated with HRP using the Lightning-Link® HRP conjugation reagents, 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.

This kit has ready-to-go assay plates, pre-coated with antibody, which are specific for the Capture Peptide.

2. Protocol Summary

Conjugate the capture and detection antibodies.

Prepare antibody mix.

Prepare samples.

Add samples and antibody mix to assay plate.
Cover and incubate at RT for 60 mins.

Wash wells then add detection reagents.
DO NOT ALLOW PLATE TO DRY.
Cover and incubate at RT for 15 mins.

Add stop solution and read absorbance at 450 nm

3. 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 handle 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.

4. Storage and Stability

- Store the buffer pack at *+4°C*.
- Store both the Capture Peptide Conjugation Kit (Capture Peptide, Capture Modifier reagent and Capture Quencher reagent) and the Lightning-Link® HRP Conjugation Kit (HRP Mix, HRP-modifier reagent and HRP-quencher reagent) at -20°C upon receipt.

  Δ Note: *The modifier and quencher, after initial thawing, can be stored at either +4°C or* -20°C*.*

  Δ Note: *Assay plates can be stored at either +4°C or room temperature.*

5. 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.

6. Materials Supplied

Item	Quantity	Storage Condition
HRP-Labeled Antibody Dilution Buffer	1 unit	+4°C
Peptide tagged Antibody Dilution Buffer	1 unit	+4°C
Detection Reagent	1 unit	+4°C
Stop Solution	1 unit	+4°C
Wash Buffer	1 unit	+4°C
Sample Dilution Buffer	2 units	+4°C
Lysis Extraction Buffer	1 unit	+4°C
Assay Plates	5 units	+4°C
Capture Peptide	3 vials	-20°C
Capture Modifier reagent	1 vial	-20°C
Capture Quencher reagent	1 vial	-20°C
HRP Mix	3 vials	-20°C
HRP-modifier reagent	1 vial	-20°C
HRP-quencher reagent	1 vial	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

9. Capture Peptide Conjugation Kit protocol:

1.1 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 buffer (e.g. MES, MOPS, 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	≤0.01
Borate buffer	Yes
Nucleophilic components (e.g. primary amines, amino acids, ethanolamine, thiols (e.g. mercatoethanol 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)*

1.2 Conjugation Protocol

This technology works by targeting amine groups (e.g. lysines) and is widely used to label antibodies, peptides, proteins and other molecules with free amine groups. The protocol provided here is optimized for labeling IgGs.

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 100 µL. We recommend not altering the reaction volume as this will alter the kinetics of the conjugation reaction.

To ensure optimal performance, we recommend conjugating

Capture Peptide to the capture antibody at 1 mg/mL.

If antibodies are used at the lower concentration (e.g. 0.2 mg/mL), excess free Capture Peptide will be present post conjugation. This excess label will be deactivated by the quencher and does not pose a problem when used on assay plates. Surplus peptide binding sites are available on assay plates to accommodate excess free label.

1.2.1 To your antibody solution, 10 µL of Capture Modifier, and mix gently.

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

1.2.3 Place the cap back on the vial and leave the vial standing for 45 minutes at room temperature (20-25°C).

1.2.4 After incubating for 45 minutes, add 10 µL of Capture Quencher and incubate for 15 minutes. No separation steps are necessary.

1.3 Storage of conjugates:

– Once conjugated, store the 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 conjugate must be determined by experimentation.

10. Lightning-Link® HRP Conjugation Kit protocol:

1.4 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 buffer (e.g. MES, MOPS, HEPES, PBS)	Yes
Non-buffering salts (e.g. NaCl)	Yes
Chelating agents (e.g. EDTA)	Yes
Sugars	Yes
Glycerol	<50%
Thiomersal / thimerosal	No
Merthiolate	No
Sodium Azide	No
BSA	<0.1%
Gelatin	<0.1%
Tris	≤50 mM
Glycine	No
Proclin	No
Borate buffer	Yes
Nucleophilic components (e.g. primary amines, amino acids, ethanolamine, thiols (e.g. mercatoethanol 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)*

1.5 Conjugation protocol

This technology works by targeting amine groups (e.g. lysines) and is widely used to label antibodies, peptides, proteins and other molecules with free amine groups. The protocol provided here is optimized for labeling IgGs.

To ensure optimal performance in the SimpleStep ELISA® Custom ELISA Kit, we recommend conjugating the antibody at a 2 mg/mL concentration (1:2 antibody:HRP).

Antibodies at concentrations between 0.5 and 4 mg/mL can still be conjugated in a fixed volume of 100 µL. Adding less than the recommended lowest amount of antibody may result in unbound label post conjugation. This excess label will be deactivated by the quencher and removed during the first wash step. Antibodies below 0.5 mg/mL should be concentrated prior to use.

Lowest amount of antibody	Recommended amount of antibody	Maximum amount of antibody	Fixed volume
50 µg at 0.5 mg/mL	200 µg at 2 mg/mL	400 µg at 4 mg/mL	100 µL

1.5.1 Before you add antibody to the HRP Mix, add 10 µL of HRP-Modifier reagent to the antibody and mix gently.

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

1.5.3 Place the cap back on the vial and leave the vial standing for 3 hours at room temperature (20-25□C). 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.

1.5.4 After incubating for 3 hours (or more), add 10 µL of HRP-Quencher. The conjugate can be used after 30 minutes. No separation steps are necessary.

1.6 Storage of conjugates:

- HRP Conjugate Stabilizer - LifeXtend (ab270548) is a proprietary multi-component reagent system that protects antibody-HRP conjugates thus ensuring the best possible performance in experiments performed at room temperature.

11. Capture and Detection Antibody Preparation:

- The capture antibody conjugated to Capture Peptide should be diluted in the 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-Labeled Antibody Dilution Buffer just prior to assaying and needs to be 4x the desired final concentration.
- The optimal working concentrations of both Peptide tagged Antibody and HRP-Labeled Antibody should be determined by titration. The final concentration in the well is typically, 100-500 ng/mL and 50-250 ng/mL in 100 µL for Peptide tagged Antibody and HRP-Labeled Antibody, respectively.

1.7 Antibody Cocktail Preparation:

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

12. 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_2O and Lysis Extraction Buffer (5X) (e.g. 4mL H_2O + 1mL of Lysis Extraction Buffer (5X)).
- Prepare 1X Wash Buffer by mixing H_2O and Wash Buffer (10X) in a 9:1 ratio (e.g. 9mL H_2O + 1mL Wash Buffer (10X)). For a single 8-well strip, ~5mL 1X Wash Buffer is needed.
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The following protocols require the preparation of cellular lysates in a separate 96 well tissue culture microplate. The lysate is subsequently transferred to an Assay Plate for the assay.

1.8 Lysate preparation – Adherent cells:

1.8.1 Remove any media and cellular treatments from the cells.

1.8.2 Optional: Wash cells with PBS.

1.8.3 Lyse the cells with 100µL/well of 1X Lysis Extraction Buffer, under shaking (~300 rpm) 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.

1.9 Lysate preparation – Non adherent cells:

1.9.1 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.

1.9.2 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.

1.9.3 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)).

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

1.10 Prepare all reagents, working standards, and samples as directed in the previous sections.

1.11 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

1.12 Add 50 µL of sample to appropriate wells.
Optional: Add 50 µL/well of Sample Dilution Buffer or 1X Lysis Extraction Buffer to separate wells to assess the background signal.

1.13 Add 50 µL of Antibody Cocktail to each well.

1.14 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to ~400 rpm.

1.15 Wash each well with 200 µL of 1X Wash Buffer 3 times. Wash by aspirating or decanting from wells then dispensing 200 µL 1X Wash Buffer into each well. Wash Buffer should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

1.16 Add 100 µL of Detection Reagent to each well and incubate for 15 minutes in the dark at room temperature on a plate shaker set to ~300 rpm.
Δ Note: *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.*
Δ Note: *Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.*
Δ Note: *The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.*

1.17 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 min at 400 rpm. Read absorbance at 450 nm.

1.18 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength	600 nm
Time	Up to 20 min
Interval	20 sec – 1 min
Shaking	Shake between readings

*Δ **Note:** An endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm. 13.10 Analyze the data as described below.*

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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