

ab309392 – Human Desmoglein-1 ELISA Kit

For the quantitative measurement of Desmoglein-1 in Human Serum, Plasma and Cell culture supernatants.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab309392>

Storage and Stability

The entire ELISA kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Materials Supplied

Item	Quantity	Storage Condition
20X Wash Buffer	25 mL	-20°C
450X HRP-Streptavidin	200 µL	-20°C
5X Assay Diluent B (Item E)	15 mL	-20°C
5X Assay Diluent D (Item K)	15 mL	-20°C
Assay Diluent A (Item D)	30 mL	-20°C
Biotinylated Human Desmoglein-1 Detection Antibody	2 vials	-20°C
Human Desmoglein-1 Antibody-coated ELISA Plate	1 unit	-20°C
Lyophilized Human Desmoglein-1 Protein Standard	2 vials	-20°C
Stop Solution	8 mL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions.

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

5X Assay Diluent D and 5X Assay Diluent B: Should be diluted 5-fold with deionized or distilled water before use.

Sample dilution: Assay Diluent A should be used for dilution of serum and plasma samples. 1X Assay Diluent D (Item K) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold.

Note: Levels of Desmoglein-1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Detection Antibody Desmoglein-1: Briefly spin the vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently

(the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B.

20X Wash Buffer: If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

450X HRP-Streptavidin: Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 450-fold with 1X Assay Diluent B.

Briefly spin the vial and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin into a tube with 9 ml 1X Assay Diluent B to prepare a 450-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

Standard Preparation

Always prepare a fresh set of standards for every use.

Discard working standard dilutions after use as they do not store well.

The following section describes the preparation of a standard curve for duplicate measurements (recommended).

- Briefly spin the Protein Standard.
- Add 400 µL Assay Diluent A (for serum/plasma samples) or Assay Diluent D (for cell culture medium, Assay Diluent D should be diluted 5-fold with deionized or distilled water before use) into the protein standard vial to prepare a 200 ng/ml standard solution. Gently mix the powder to allow it to dissolve thoroughly.
- Pipette 270 µL Assay Diluent A or 1X Assay Diluent D into each tube. Use the standard solution to produce a dilution series. Adding 180 µL from #1 to #2, then from #2 to #3, etc.
- Mix each tube thoroughly before the next transfer.
- Tube #8 contains no protein and is the Blank control.

Tube #	Volume to dilute	Volume of 1X Assay Diluent	Final Concentration ng/mL
1	200 ng/mL standard stock solution	-	200
2	180 µL of tube #1	270 µL	80
3	180 µL of tube #1	270 µL	32
4	180 µL of tube #1	270 µL	12.8
5	180 µL of tube #1	270 µL	5.12
6	180 µL of tube #1	270 µL	2.048
7	180 µL of tube #1	270 µL	0.819
8	-	270 µL	0

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

1. Label removable 8-well strips as appropriate for your experiment.
2. Add 100 μ L of standard or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature with gentle shaking.
3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 μ L) using a multichannel Pipette or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μ L of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 μ L of prepared Streptavidin solution each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 μ L of TMB Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 μ L of Stop Solution; 0.2M Sulfuric Acid to each well. Read at 450 nm immediately.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Technical Support

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