# Anti-Desmoglein1 ELISA kit

MBL-7881R : 48 wells

FOR RESEARCH USE ONLY

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

**[INTENDED PURPOSE]**

Anti-Desmoglein1 ELISA kit is for semi-quantitative measurement of anti-Desmoglein 1 (Dsg1) antibodies in human serum by *ELISA.

*ELISA: Enzyme-Linked Immunosorbent Assay

**[ASSAY PRINCIPLE]**

Anti-Desmoglein1 ELISA kit measures anti-Dsg1 antibodies present in serum sample. Calibrators and samples are added to the microwells coated with Dsg1. During sample incubation, anti-Dsg1 antibodies will react with the immobilized antigen (Sample incubation). After wash to remove any unbound serum proteins, horseradish peroxidase conjugated anti-human IgG polyclonal antibody is added and incubated (Conjugate incubation). After another washing step, the peroxidase substrate is added and incubated for an additional period of time (Substrate incubation). Acid solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The test result is determined photometrically by measuring the absorbance.

**[REAGENTS AND STORAGE]**

| Product Code | Product Name | Quantity |
|---|---|---|
| MBL-7881R | Anti-Desmoglein1 ELISA kit | 48 wells |

| Kit Component | Quantity |
|---|---|
| **Dsg1 Microwell strips**<br>Microwells coated with recombinant Dsg1. The breakaway strips are packed in a strip holder and sealed in a foil envelope with a desiccant. Ready to use. | 8 wells x 6 strips |
| **Calibrator 1 (0 U/mL)**<br>Containing human serum and 0.09% sodium azide. Ready to use. | 1.5 mL x 1 vial |
| **Dsg1 Calibrator 2 (100 U/mL)**<br>Containing anti-Dsg1 antibody positive human serum and 0.09% sodium azide. Ready to use. | 1.5 mL x 1 vial |
| **Conjugate reagent**<br>Containing horseradish peroxidase conjugated goat anti-human IgG antibody and 0.15% *ProClin 150. Ready to use. | 15 mL x 1 vial |
| **Assay diluent**<br>Containing bovine serum and 0.09% sodium azide. Ready to use. | 50 mL x 1 vial |
| **Wash concentrate (20x)**<br>Containing phosphate with Tween 20, as a 20x concentrate. | 50 mL x 1 vial |
| **Substrate**<br>Containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. | 20 mL x 1 vial |
| **Stop solution**<br>Containing 0.25 mol/L sulfuric acid. Ready to use. | 20 mL x 1 vial |
| **Control I**<br>Containing human serum and 0.09% sodium azide. Ready to use. | 1.5 mL x 1 vial |
| **Control II**<br>Containing anti-Dsg1 antibody positive human serum and 0.09% sodium azide. Ready to use. | 1.5 mL x 1 vial |

*ProClin 150: reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

Anti-Desmoglein1 ELISA kit shall be kept at 2-8°C.
The expiration date is provided on the labels. Do not use expired components.
After first opening, the kit components are stable for up to 3 months at 2-8 °C.

**[REQUIRED MATERIALS AND DEVICES NOT SUPPLIED]**

- Microplate reader (wavelength: 450 nm, 620 nm/reference)
- Multichannel micropipette (e.g. 100 µL - 300 µL)
- Single channel pipette (e.g. 10 µL & 100 µL)
- Reagent reservoir
- Autowasher or wash bottle
- Deionized or distilled water
- One liter graduated cylinder for preparation of Wash solution
- Test tubes for sample dilution (e.g. 1,000 µL)
- Disposable pipette tips
- Paper towels
- Microplate cover

**[WARNINGS AND PRECAUTIONS]**

(1) This product is for research use only. Do not use in diagnostic procedures.
(2) Do not use the kit components beyond the stated expiration dates.
(3) Avoid contact of reagents with eyes, skin and clothing. Reagents on skin must be washed away with plenty of water.
(4) Calibrator 1 (0 U/mL), Dsg1 Calibrator 2 (100 U/mL), Control I and Control II contain human serum, in which HBs antigen, HCV antibody HIV-1 and HIV-2 antibodies have not been detected. No test method, however, can guarantee the absence of these or any other infectious substances. These kit components and samples should be handled as they are capable of transmitting AIDS, hepatitis or any other infectious diseases.
(5) Calibrator 1 (0 U/mL), Dsg1 Calibrator 2 (100 U/mL), Assay diluent, Control I and Control II contain sodium azide (0.09%) as a preservative and must be handled with caution. Do not ingest or allow contact with skin or mucous membranes. Sodium azide may react with copper or lead in plumbing system to form explosive metal azides. Collect and reclaim or dispose in sealed containers at licensed waste disposal site. Dispose of contents/containers in accordance with local/regional/national/international regulations. Avoid release to environment.
(6) Conjugate reagent contains ProClin 150 (0.15%) as a preservative and must be handled with caution. Do not ingest or allow contact with skin or mucous membranes. ProClin 150 may cause an allergic skin reaction.
(7) Substrate contains TMB. TMB may cause irritation or dryness of the skin.

(8) Stop solution contains sulfuric acid (0.25 mol/L) and must be handled with caution. Sulfuric acid may cause severe corrosive skin damage.

(9) Some kit components contain materials of animal origin. These components should be treated as potential biohazards in use and for disposal.

(10) Matching lot numbers of Dsg1 Microwell strips, Conjugate reagent and Dsg1 Calibrator 2 (100 U/mL) must be used together in the assay. Do not mix reagents from other kits.

(11) All reagents must be brought to room temperature (20-30°C) before starting the assay.

(12) Do not expose the kit to direct sunlight during assay and storage.

(13) Avoid microbial and cross contamination of reagents or samples.

(14) Incubation temperatures above or below normal room temperature (20-30°C), shorter or longer incubation times and inaccurate dilution may give erroneous results.

(15) The wells must be properly rinsed with Wash solution to avoid false positive.

(16) Carefully pipette each sample and reagent to avoid cross contamination between microwells, avoid foaming.

(17) Assay diluent may form precipitates, which does not cause inconsistent results.

(18) Wash concentrate (20x) may become turbid at 2-8°C, this does not cause inconsistent results. The precipitates should be dissolved thoroughly and then diluted to prepare Wash solution.

(19) Materials used for the assay should be disposed or treated as shown below. Soak in 2% final conc. glutaraldehyde solution for more than 1 hour, soak in 0.1% Sodium hypochlorite solution (available chlorine: approx. 1,000 ppm) for more than 1 hour or autoclave at 121°C for more than 20 minutes.

(20) In the event of damage to the protective packaging, the kit components should be treated or disposed of in accordance with the relevant instructions and regulations of each country.

(21) All unused Dsg1 Microwell strips should be returned to the pouch, which must be carefully resealed to avoid moisture absorption. Reagent bottles are stored in an upright position, with caps tightly closed.

(22) Equipments and instruments used for this product must be accuracy controlled and maintained.

**[PROCEDURE]**

■ **PREPARATION OF REAGENTS**

- Bring all assay materials to room temperature (20-30°C) prior to use.
- Microwell strips: Remove required Microwell strips from the pouch and place them in the frame. Promptly return unused strips to refrigerated storage.
- Wash solution: Wash concentrate (20x) must be diluted by 20 times prior to use. Prepare the required amount of Wash solution, for example, add 50 mL of Wash concentrate (20x) to 950 mL of distilled water. The diluted Wash solution is stable for 14 days at 2-8°C.
- Do not dilute Calibrator 1 (0 U/mL), Dsg1 Calibrator 2 (100 U/mL), Conjugate reagent, Assay diluent, Substrate, Stop solution, Control I and Control II. These reagents are ready to use.

## ■ PREPARATION OF SAMPLES

- Use fresh sera. Samples should be tested as soon as possible after collection. If storage is needed, they should be stored at -20°C or lower and used within four weeks. Do not repeat freezing and thawing.
- Dilute each serum 1:101 by adding 10 µL of serum to 1000 µL of Assay diluent.
  *Diluted sample must be stored at 4°C and used within three days.
  *Diluted samples can be also used for Anti-Desmoglein3 ELISA kit.
- Highly hemolysed samples or highly lipemic samples should not be used.
- Samples should not be heat-inactivated.

## ■ ASSAY PROCEDURE

This device is used for manual testing (not automated).

**STEP 1. (SAMPLE INCUBATION)**

Transfer 100 µL of Calibrator 1, Calibrator 2, each diluted sample, and if necessary, Control I and Control II, with a multichannel micropipette, into the appropriate microwells of Dsg1 Microwell strips. Do not dilute Calibrator1, Calibrator 2, Control I and Control II.

*Reaction starts on pipetting into the antigen-coated microwells. Pipetting should be completed as quickly as possible.

Cover the wells with a microplate cover and incubate at room temperature (20-30°C) for 60 minutes.

**STEP 2. (WASH)**

Aspirate or discard the well contents. Fill the well with Wash solution and then completely aspirate or discard the contents. Wash 4 times. Tap the plate on a paper towel to remove any remaining Wash solution. When autowasher is used, wash 4 times.

*Each laboratory is recommended to confirm its own appropriate washing times and set-up.

*Wash solution should be used at 20-30°C.

**STEP 3. (CONJUGATE INCUBATION)**

Pour Conjugate reagent into a reservoir. Add 100 µL of Conjugate reagent to each well with a multichannel micropipette. Cover the wells with a microplate cover and incubate at room temperature (20-30°C) for 60 minutes.

*Conjugate reagent, once put in a reservoir, should not be returned to the vial.

**STEP 4. (WASH)**

Wash the well following STEP 2 procedure.

**STEP 5. (SUBSTRATE INCUBATION)**

Pour Substrate into a reservoir. Add 100 µL of Substrate to each well with a multichannel micropipette.

*This reservoir should be different from the one, used for distributing Conjugate reagent. A new disposable reservoir should be used because Substrate is easily oxidized by metal ion.

*Substrate, once put in a reservoir, should not be returned to the vial.

Cover the wells with a microplate cover and incubate at room temperature (20-30°C) for 30 minutes.

**STEP 6. (STOP REACTION)**

Pour Stop solution into a reservoir. Add 100 µL of Stop solution to each well with a multichannel micropipette.

**STEP 7. (READING)**

Read the absorbance of each well at 450 nm. If a dual wave length plate reader is used, set the test wavelength at 450 nm and the reference at 620 nm.

*Reading should be done as quickly as possible after stopping the reaction.

*Before reading the plate, ensure that the bottom of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells.

**STEP 8. (CALCULATION)**

For results calculated by reader program or manually, use the following formula:

$$\text{Unit value (U/mL)} = \frac{(A_{450}\text{<Sample>} - A_{450}\text{<Calibrator 1 (0 U/mL)>})}{(A_{450}\text{<Dsg1 Calibrator 2 (100 U/mL)>} - A_{450}\text{<Calibrator 1 (0 U/mL)>})} \times 100$$

*$A_{450}$ is an abbreviation of absorbance value at 450 nm.

*An international reference material for anti-Dsg1 antibody is not available. The assay is calibrated in relative arbitrary units.

*There is a possibility that the antibody titer is not caught clearly when the antibody value is higher than 150 U/mL. The antibody titer can be caught more clearly by measuring the sample by a higher dilution (ex.1: 2,020 dilution).

*Conversions calculated from further dilutions of a sample may not be accurate, as some samples do not have good dilution linearity.

**BRIEF ASSAY PROCEDURE**

| | |
|---|---|
| <SAMPLE INCUBATION> | Add 100 µL of diluted sample (1:101) to each well. |
| ↓ | 20-30°C, 60 min. |
| <WASH> | |
| ↓ | |
| <CONJUGATE INCUBATION> | Add 100 µL of Conjugate reagent to each well. |
| ↓ | 20-30°C, 60 min. |
| <WASH> | |
| ↓ | |
| <SUBSTRATE INCUBATION> | Add 100 µL of Substrate to each well. |
| ↓ | 20-30°C, 30 min. |
| <STOP REACTION> | Add 100 µL of Stop solution to each well. |
| ↓ | |
| <READING> | |
| <CALCULATION> | |
| <INTERPRETATION> | |

**[PERFORMANCE CHARACTERISTICS]**

■ ANALYTICAL PERFORMANCE

(1) PRECISION OF MEASUREMENT

1) REPEATABILITY

When two samples were measured in sextuplicate three times using three lots of Anti-Desmoglein1 ELISA kit, the CV of the measured values was within 15%.

2) INTER-DAY REPRODUCIBILITY

When five samples were tested for nine days using one lot of Anti-Desmoglein1 ELISA kit, the CV of the measured values was within 15%.

3) LOT-TO-LOT REPRODUCIBILITY

When six samples were tested three times each using three lots of Anti-Desmoglein1 ELISA kit, the CV of the measured values was within 15%.

4) INTER-ANALYST REPRODUCIBILITY

When four samples were tested once each by three analysts using one lot of Anti-Desmoglein1 ELISA kit, the measured values were within ±15% of the expected value.

(2) ANALYTICAL SENSITIVITY

The LOQ given as the CV of 15% was 0.65 U/mL.

(3) ANALYTICAL SPECIFICITY

When samples were spiked with co-substances up to the following concentrations for measurement, the measured values were within ±15% of the value measured in non-spiked samples, and no certain relations were observed between the measured values and co-substance concentrations.

Bilirubin F (free bilirubin) : 19.6 mg/dL
Bilirubin C (conjugated bilirubin) : 21.6 mg/dL
Hemoglobin: 476.0 mg/dL
Chyle: 1410 formazin turbidity unit (FTU)
Rheumatoid factor (RF): 1000 IU/mL

(4) MEASURING RANGE OF THE ASSAY

1) UPPER LIMIT

150 U/mL

2) LOWER LIMIT

5 U/mL

3) PROZONE

No prozone phenomenon was observed in the range up to 2000 U/mL.

**[QUALITY CONTROL]**

Each assay result should meet the following criteria.

$A_{450}$ of Calibrator 1 (0 U/mL) : $\leq 0.100$

$A_{450}$ of Dsg1 Calibrator 2 (100 U/mL) : $\geq 0.500$

When Control I and Control II are measured, each measured value must be within the concentration range indicated on the respective labels.

If any of these criteria are not met, the results are invalid and the test should be repeated.

Before repeating assay, check the following points.

- Incubation Temperature
- Incubation Time
- Washing conditions

The values for Calibrator 1, Calibrator 2, Control I and Control II have been set in-house.

**[MANUFACTURER]**

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**[REVISION]**

January 30, 2026
No. 7881R-0