

MBL

CE

MESACUP Desmoglein TEST “Dsg3”

Cat. No. 7685E: 48 wells

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

5-10 Marunouchi 3 chome, Naka-ku, Nagoya, 460-0002 Japan

Tel: +81 52-971-2081 Fax : +81 52-971-2337 URL <http://www.mbl.co.jp>

English

INTENDED USE

The MESACUP Desmoglein TEST “Dsg3” is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Desmoglein3 antibodies in human serum. The MESACUP Desmoglein TEST “Dsg3” is intended for in vitro diagnostic use as an aid in the differential diagnosis of certain pemphigus diseases.

SUMMARY AND EXPLANATION

Desmoglein 3 (Dsg3) is a desmosomal cadherin expressed in stratified squamous epithelia and the target antigen in pemphigus vulgaris (PV) [1-3]. PV is a fatal autoimmune blistering disease of the skin and mucous membrane and clinically characterized by flaccid blisters and widespread painful erosions. Histologically, the lesions of PV demonstrate intraepidermal blister formation due to loss of cell adhesion of keratinocytes. Anti-Dsg3 autoantibodies (IgG) are detected in sera from patients with PV and play a pathogenic role in blister formation in PV. Anti-Dsg3 IgG is not found in patients with pemphigus foliaceus (PF), another type of pemphigus. Amagai et al have produced a recombinant human Dsg3 (rDsg3) using a baculovirus expression system [4]. rDsg3 is designed as a secreted protein consisting of the entire extracellular domain of Dsg3. rDsg3 is capable of immunoadsorbing pathogenic autoantibodies in PV patients' sera, preventing blister formation in a neonatal mouse model of pemphigus. These findings indicate that rDsg3 expresses most, if not all, conformational epitopes of the native antigens critical in the pathogenesis of pemphigus. Currently, diagnosis of pemphigus largely relies on immunofluorescence testing (IF) utilizing sectioned normal human skin or monkey esophagus. However, there are several disadvantages in the current immunofluorescence. (a) IF requires an experienced examiner and a subjective determination of antibody titer. (b) Antibody against any cell surface protein makes false positive. (c) Similar staining pattern in IF makes it difficult to distinguish between PV and PF. To overcome the problems of IF, ELISA has been developed using rDsg3 as a sensitive and specific assay for the diagnosis of pemphigus [5].

The MESACUP Desmoglein TEST “Dsg3” is for measuring anti Desmoglein 3 IgG autoantibodies with high sensitivity.

PRINCIPLE

MESACUP Desmoglein TEST “Dsg3” measures anti-Dsg3 antibodies present in the serum by ELISA. Calibrators and patient sera are added to microwell coated with Dsg3, allowing anti-Dsg3 antibodies to react with the immobilized antigen (Sample incubation). After wash to remove any unbound serum proteins, horseradish peroxidase conjugated anti-human IgG monoclonal antibody is added and incubated (Conjugate incubation). Following 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 assay can be quantified by measuring the reaction photometrically.

BRIEF ASSAY PROCEDURE

<Sample incubation> (20-25°C) 60 min.	Add 100µl of diluted sample (x101) to each well of microwell plate. ↓ Wash ↓
<Conjugate incubation> (20-25°C) 60 min.	Add 100µl of conjugate solution to each well. ↓

Wash
↓
<Substrate incubation> Add 100µl of substrate to each well.
(20-25°C) 30 min. ↓
Add 100µl of stop solution to each well.
↓
Read absorbance
↓
Interpretation of result

REAGENTS AND STORAGE

1) Dsg-3 MICROWELL STRIPS

48 wells MICROWELL STRIPS (6 x 8 wells) coated with recombinant purified Dsg-3 antigen, the breakaway strips packed in a strip holder and sealed in a foil envelope with desiccant, are stable at 2-8°C until labeled expiration date.

2) Calibrator 1 (0 U/ml)

One vial containing 1.5ml of normal human serum with Assay Diluent including 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

3) Dsg-3 Calibrator 2 (100U/ml)

One vial containing 1.5ml of anti Dsg-3 antibody positive human serum with Assay Diluent including 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

4) Conjugate Reagent (101x)

One vial containing 0.3ml of horseradish peroxidase conjugated mouse monoclonal anti human IgG at a 101x concentration. Stable at 2-8°C until labeled expiration date.

5) Conjugate Diluent

One vial containing 24ml of HEPES and bovine serum albumin. Stable at 2-8°C until labeled expiration date.

6) Assay Diluent

One vial containing 50ml of Tris buffer, bovine serum and 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

7) Wash Concentrate (10x)

One vial containing 100ml of PBS and Tween 20 as a 10x concentrate. Stable at 2-8°C until labeled expiration date.

8) Substrate

One vial containing 20ml of 3,3',5,5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H₂O₂). Stable at 2-8°C until labeled expiration date.

9) Stop Solution

One vial containing 20ml of 1.0N sulfuric acid. Stable at 2-8°C until labeled expiration date

PRECAUTIONS

- (1) This product is for in vitro diagnostic use only.
- (2) Do not use 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. TMB contains irritant and Stop Solution consists of a 1N sulfuric acid, which is a poison and corrosive.
- (4) Calibrators are derived from human serum, in which HBs antigen, HCV antibody HIV-1 and HIV-2 antibodies has not been detected. No test method, however, can guarantee the absence of these or any other infectious agents. These reagents and all patient samples should be handled as if they are capable of transmitting AIDS, hepatitis or any other infectious diseases.
- (5) Calibrator 1, Calibrator 2 and Assay Diluent 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. Therefore, always flush with plenty of water when disposing materials containing sodium azide into a drain.
- (6) Some kit components contain animal origin materials, which are from non-infectious animals. These components, however, should be treated as potential biohazards in use and for disposal.
- (7) Matching lot numbers of Microwell strips, Conjugate and Calibrator 2 must be used together in the assay. Do not substitute reagents from other kits.
- (8) All reagents must be brought to room temperature (20-25°C) before starting the assay.
- (9) Do not expose the kit to direct sun during assay and storage.
- (10) Avoid microbial and cross contamination of reagents or samples.
- (11) Incubation temperatures above or below normal room temperature (20-25°C), shorter or longer time periods of incubation and inaccurate dilution may give erroneous results.
- (12) The wells must be rinsed with Wash Solution properly enough to avoid false positive.
- (13) Carefully pipette not to foam each sample and reagent to avoid cross contamination between microwells.
- (14) All microwell strips, which are not immediately required, should be returned to the zip lock pouch, which must be carefully resealed to avoid moisture absorption.
- (15) Wash concentrate may become turbid at 2-8°C, which does not cause inconsistent results.
- (16) Implement used for the test should be disposed or treated as shown below.

Soak in 2% final conc. glutaraldehyde solution for more than 1 hour or soak in 0.5% Sodium hypochlorite solution (available chlorine: approx. 5000ppm) for more than 1 hour or autoclave at 121°C for more than 20 minutes.
- (17) The Dsg-3 antibodies value obtained from this assay are an aid to diagnosis only. Each physician must interpret these results in light of the patient's history, physical findings, and other diagnostic procedure.

MATERIALS REQUESTED BUT NOT PROVIDED

- Microplate reader(wavelength: 450nm, 620 nm/reference)
- Multichannel micropipette (e.g. 100µl – 300µl)

- Single channel pipette (10 μ l & 100 μ l)
- Reagent reservoir
- Autowasher or wash bottle
- Deionized or distilled water
- One liter graduated cylinder for preparation of wash solution
- Test tubes for patient sample dilutions (e.g. 1000 μ l)
- Disposable pipette tips
- Paper towels
- Basin and disinfectant
- Microplate cover

PROCEDURE

■ PREPARATION OF REAGENTS

- Bring all assay materials to room temperature(20-25°C) prior to use.
- Microwell Strips: Remove required microwell strip from pouch and place them in the frame. Promptly return unused strips to refrigerated storage.
- Wash Solution: The Wash Concentrate must be diluted prior to use. Dilute the Wash Concentrate 1:10 by adding 50mL of the concentrate to 450mL of distilled water. The diluted wash solution is stable for 2 weeks at 2-8°C.
- Conjugate Solution: The conjugate reagent must be diluted prior to use. Dilute conjugate 1:101 with Conjugate Diluent. The diluted conjugate solution must be used within a day.
- Use disposable reservoir to avoid contamination.
- Do not dilute the other kit components which are ready- to-use.

■ PREPARATION OF SAMPLES

- Use fresh patient sera. If storage is needed, they should be aliquot and frozen below -20°C for up to one month, below -70°C for longer storage. Do not repeat freezing and thawing.
 - *In case stored below -20°C for more than 6 months or freezing and thawing repeatedly, nonspecific results are obtained because of IgG denaturation.
- Dilute each patient serum 1:101 by adding 10 μ l of serum to 1ml of Assay Diluents.
 - *Diluted samples may be stored up to 3 days if refrigerated.
 - *Diluted samples can be also used for MESACUP Desmoglein TEST "Dsg1".

■ ASSAY PROCEDURE

STEP 1. (SAMPLE INCUBATION)

Pipette 150 μ l Calibrator 1, Dsg3 Calibrator 2 and each diluted sample to the appropriate polyvinyl plate well respectively, then pipette 100 μ l each with multichannel pipette into the appropriate microwell coated with antigen.

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

Cover wells with a plate sealer and incubate at room temperature (20-25°C) for 60 minutes.

STEP 2. (WASHING)

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.

* Washing Solution should be used at 20-25°C.

STEP 3. (CONJUGATE INCUBATION)

Pour Conjugate Solution into a reservoir. Add 100µl of the working Conjugate Solution to each well with multichannel pipette. Cover wells with a plate sealer and incubate at room temperature (20-25°C) for 60 minutes.

STEP 4. (WASHING)

Wash the microplate following the STEP 2 procedure.

STEP 5. (SUBSTRATE INCUBATION)

Pour substrate into a reservoir. Add 100µl of the substrate to each well with multichannel pipette.

* This reservoir should be different from the one, which was used for pouring conjugate solution. A new disposable reservoir should be used because Substrate is easily oxidized by metal ion. Pipette 100µl of the substrate to each well with multichannel pipette.

Cover wells with a plate sealer and incubate at room temperature (20-25°C) for 30 minutes.

STEP 6. (STOP REACTION)

Pour Stop solution into a reservoir. Add 100µl of the solution to each well with multichannel pipette.

■ READING

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

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

*Ensure that the bottom of the plate is clean and dry, and that no bubbles are present on the surface of the liquid of the wells before reading the plate.

■ CALCULATION OF RESULTS

$$\text{Unit value (U/ml)} = \frac{(A_{450}\langle\text{Sample}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)}{(A_{450}\langle\text{Calibrator 2}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)} \times 100$$

*A450 is abbreviation of absorbance value at 450nm.

*An international reference material for anti Dsg-3 antibody is not available, the assay is calibrated in relative arbitrary units.

■ QUALITY CONTROL

Each assay result should meet the following criteria.

$$A_{450} \text{ of Calibrator1: } \leq 0.100$$

$$A_{450} \text{ of Dsg3 Calibrator2: } \geq 0.700$$

If any of these are met, the results are invalid and the test should be repeated.

Before repeating assay, check the following procedure.

- Incubation Temperature
- Incubation Period of Time
- Washing

TEST INTERPRETATION AND EXPECTED VALUE

The following is intended only as a guide for interpretation. Each laboratory is recommended to establish its own criteria for test interpretation based on sample populations typically encountered.

Anti-Dsg3 value (U/ml)	Interpretation
≥ 20	Positive
$20 > - \geq 7$	Indeterminate
$7 >$	Negative

- The above value was established with assaying 179 normal serums, 60 Pemphigus vulgaris(PV) patient serums and 246 patient serums of other than PV.
- When it is interpreted as Indeterminate, a closer look at transition of the disease is recommended.
- This reagent shows antibody titer as assay value. A transition of antibody titer is thought to reflect disease activity of patient, but there is a possibility that the change of the antibody titer is not caught clearly when the antibody value is higher than 150. The change of the antibody titer can be caught more clearly by measuring the specimen by a higher dilution (ex.1: 2,020 dilution).

■ LIMITATIONS

As with other diagnostic test procedures, the results obtained with the MESACUP Desmoglein TEST “Dsg3” serve only as an aid to diagnosis and should not be interpreted as diagnostics in themselves.

PERFORMANCE CHARACTERISTICS

■ CLINICAL SPECIFICITY AND CLINICAL SENSITIVITY

Disease	Positive sample	Positive rate
Pemphigus vulgaris (PV)	39/39	100.0%
Pemphigus foliaceus (PF)	0/31	0.0%
Bullous pemphigoid (BP)	1/45	2.2%
Normal Serum	0/180	0.0%

■ PRECISION

Repeatability was demonstrated by testing 3 samples in sextuple. Reproducibility was determined by testing 3 samples on 5 different days (day-to-day) and by testing 3 samples in 3 lots (lot-to-lot). %CV values for reproducibility and repeatability were below 15% for each sample.

■ ASSAY RANGE

The assay range of this kit is from 5U/ml to 150U/ml. When the assay result exceed 150U/ml should report “ over 150U/ml”.

■ INTERFERING SUBSTANCES

Hemoglobin (up to 490mg/dl), Bilirubin F (up to 18.2mg/dl), Bilirubin C (up to 22mg/dl), chyle (up to 2800 unit as Formazine) and/or Rheumatoid factor (up to 480 IU/ml) are not affective on the assay result, but avoid using highly hemorized samples or highly lipemic samples.

REFERENCES

1. Stanley JR: Cell adhesion molecules as targets of autoantibodies in pemphigus and pemphigoid, bullous diseases due to defective epidermal cell adhesion. *Adv Immunol* 53: 291-325, 1993
2. Amagai M: Pemphigus: autoimmunity to epidermal cell adhesion molecules. *Adv Dermatol* 11: 319-352, 1996
3. Amagai M, Hashimoto T, Green KJ, Shimizu N, Nishikawa T: Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. *J Invest Dermatol* 104: 895-901, 1995
4. Ishii K, Amagai M, Hall RP, Hashimoto T, Takayanagi A, Gamou S, Shimizu N, Nishikawa T: Characterization of autoantibodies in pemphigus using antigen-specific ELISAs with baculovirus expressed recombinant desmogleins. *J Immunol* 159: 2010-2017, 1997

Manufactured by:

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

5-10 Marunouchi 3-chome, Naka-ku, Nagoya 460-0002, JAPAN

Tel: +81 52-971-2081

Fax: +81 52-971-2337

Authorized Representative in the EU:

QARAD b.v.b.a.

Volmolenheide 13, 2400 Mol, Belgium

	<u>www.e-labeling.eu/MBL009323</u>
	+800 135 79 135 GR 00 800 161 220 577 99

Rev. September 9, 2009

9/9/2009

