

CE

# **MESACUP BP180 TEST**

Cat. No. 7695E: 48 wells

## English

### **INTENDED USE**

The MESACUP BP180 TEST is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti BP180 antibodies in human serum. The MESACUP BP180 TEST is intended for in vitro diagnostic use as an aid in the differential diagnosis of certain pemphigus diseases.

### **SUMMARY AND EXPLANATION**

Bullous pemphigoid (BP) is chronic itchy blistering disorder found mainly in aged person, characterized by frequent occurring of tense blister and erythema. IgG antibasement membrane zone (BMZ) antibodies are found in the serum of patients, and linear IgG or C3 sediment is found on the basement membrane zone of the lesion. Target antigens of the autoantibodies in BP patient serum are BP230 and BP180, also called BPAG1 and BPAG2. Molecular weight of these antigens is 230 kD and 180 kD respectively. BP180 is thought to be the direct target of the autoantibody because of its location, and the autoantibodies against BP230 are thought to be secondarily produced. The antibodies against BP180 are thought to be pathogenic, because the rabbit antibody against mouse NC16a region of BP180 forms blister similar to BP when injected to neonatal mice<sup>1</sup>. The main epitope of BP180 is located in the region close to cell membrane called NC16a and most of patient serum react with the recombinant NC16a protein<sup>2</sup>.

The MESACUP BP180 TEST is for measuring anti BP180 autoantibodies in patient serum specifically.

### **PRINCIPLE**

The MESACUP BP180 TEST measures anti-BP180 antibodies present in the serum by ELISA. Calibrators and patient sera are added to microwell coated with BP180 antigen, allowing anti-BP180 antibodies to react with the immobilized antigen (Sample incubation). After washing to remove any unbound serum proteins, horseradish peroxidase conjugated anti-human IgG 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> (20-30 °C) 60 min	Add 100 µl of diluted sample (1:101) to each well of microwell plate. ↓ Wash ↓
<Conjugate incubation> (20-30 °C) 60 min.	Add 100 µl of conjugate solution to each well. ↓ Wash ↓
<Substrate incubation> (20-30 °C) 30 min	Add 100 µl of substrate to each well. ↓ Add 100 µl of stop solution to each well. ↓

Read absorbance

↓

Interpretation of result

## **REAGENTS AND STORAGE**

### **1) BP180 MICROWELL STRIPS**

48 wells MICROWELL STRIPS (6 x 8 wells) coated with recombinant purified BP 180NC16a 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 (0U/ml)**

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

### **3) Calibrator 2 (100U/ml)**

One vial containing 1.5ml of anti BP180 antibody positive human serum with Assay Diluent including 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

### **4) Conjugate Reagent**

One vial containing 8ml of horseradish peroxidase conjugated goat anti human IgG. Stable at 2-8°C until labeled expiration date.

### **5) Assay Diluent**

One vial containing 50ml of PBS, Tween 20 and 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

### **6) 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.

### **7) Substrate**

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

### **8) 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, 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 -30°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 (20-30°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 BP-180 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 REQUIRED BUT NOT PROVIDED**

- Microplate reader(wavelength: 450nm, 620 nm/reference)
- Multichannel micropipette (e.g. 100µl – 300µl)
- Single channel pipette (10µl & 100µl)
- 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.1000µl)
- Disposable pipette tips
- Paper towels
- Basin and disinfectant
- Microplate cover

## **PROCEDURE**

### **■ PREPARATION OF REAGENTS**

- Bring all assay materials to room temperature(20-30°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.
- 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 any longer storage. Do not repeat freezing and thawing.
  - \*In case of being 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 10µl of serum to 1ml of Assay Diluent.
  - \*Diluted samples must be used within a day.

### **■ ASSAY PROCEDURE**

#### **STEP 1. (SAMPLE INCUBATION)**

Pipette 150 µl of Calibrator 1, Calibrator 2, and each diluted sample to the appropriate polyvinyl plate well, then pipette 100 µl 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 (20-30°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 another conditions.

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

#### **STEP 3. (CONJUGATE INCUBATION)**

Pour Conjugate solution into a reservoir. Add 100 µl of working Conjugate solution to each well with multichannel pipette. Cover wells with a plate sealer and incubate at room temperature (20-30°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 100 µl of 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.

Cover wells with a plate sealer 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 the solution to each well with multichannel pipette.

#### **■ READING**

Read the absorbance of each well at 450 nm. If a dual wavelength platereader is available, 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.

\*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 before reading the plate.

#### **■ CALCULATION OF RESULT**

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

\*A<sub>450</sub> is abbreviation of absorbance value at 450 nm.

\*An international reference material for anti BP180 antibodies is not available, the assay is calibrated in relative arbitrary units.

#### **■ QUALITY CONTROL**

Each assay result should meet the following criteria.

$$A_{450} \text{ of Calibrator 1: } \leq 0.100$$

$$A_{450} \text{ of Calibrator 2: } \geq 0.500$$

IF any of these are not 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 value was determined by ROC analysis with 64 BP samples and control samples (42 PF patients, 69 PV patients, and 336 normal individuals).

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- BP180 value (U/ml)	Interpretation
< 9	Negative for anti-BP180 Ab
≥ 9	Positive for anti-BP180 Ab

#### **■ LIMITATIONS**

As with other diagnostic test procedures, the results obtained with the MESACUP BP180 TEST 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)	0/69	0.0%
Pemphigus foliaceus (PF)	0/42	0.0%
Bullous pemphigoid (BP)	54/64	84.4%
Normal individuals	5/336	1.5%

**■ 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 7 U/ml to 150U/ml

When the assay result exceed 150U/ml should report “ over 150U/ml”.

**■ INTERFERING SUBSTANCES**

Hemoglobin (up to 440 mg/dl), Bilirubin (up to 40.0mg/dl), chyle (up to 2,350 unit as Formazine) and/or Rheumatoid factor (up to 1,000 IU/ml) are not affective on the assay result, but avoid using highly hemolysed samples or highly lipemic samples.

**REFERENCES**

1. Liu Z, et al.: A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. J Clin Invest 92:2480-2488, 1993.
2. Matsumura K, et al.: The majority of bullous pemphigoid and herpes gestationis serum samples react with NC16a domain of the 180kD bullous pemphigoid antigen. Arch Dermatol Res 288:507-509, 1996.

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