Version 15-04/25

User's Manual

Benzonase® ELISA Kit

REF **DEIA-JY2103**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Benzonase® kit is used for detection and quantitative determination of Benzonase® endonuclease in intermediates, semi-finished products and finished products of various biological products.

Principles of Testing

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A polyclonal antibody specific for Benzonase has been pre-coated onto well plate strips. Standards and samples are added to the wells and Benzonase present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Benzonase antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Benzonase bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm.

Reagents And Materials Provided

1. Benzonase Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with mouse mAb antibody against Benzonase.
2. Benzonase Standard: 1×300 µL, 0.5 µg/mL. Recombinant Benzonase in a buffer.
3. HRP-Conjugate Benzonase Antibody: 15 mL, rabbit mAb antibody against Benzonase conjugated to horseradish peroxidase (HRP) with preservatives.
4. Wash Buffer 20×: 30 mL of a 20-fold concentrated solution.
5. Sample Dilution: 30 mL
6. Chromogen Solution A: 8 mL
7. Chromogen Solution B: 8 mL
8. Stop Solution: 15 mL
9. 3 Cover foil

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Incubator 37°C
3. Shaker
4. Precision pipettes to deliver 0.5 µl to 1 ml volumes
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis

8. Tubes to prepare the positive control or sample dilutions

Storage

1. Store all reagents at 2-8°C, and not be frozen or thawed. The product is valid for 12 months
2. All reagents must be brought to room temperature (20-25°C) prior to use.

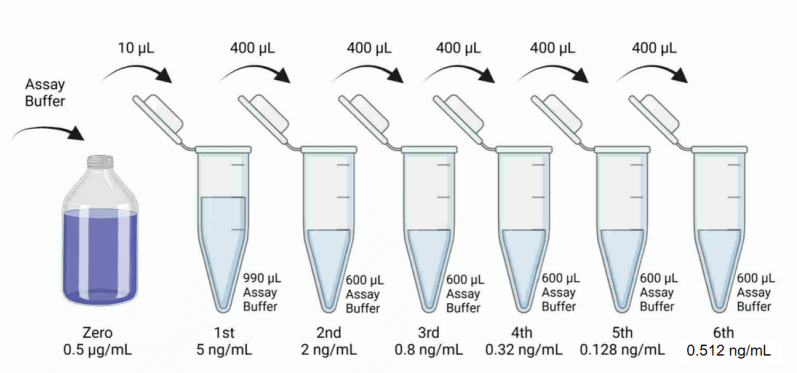
Reagent Preparation

1. Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.
2. Wash Buffer: Prepare 1× wash buffer by adding 19 parts of dcionized water to make working concentration wash buffer (1×). If any crystallization is formed in wash buffer (20×), it should be placed at room temperature or shaken gently in 37 water bath. Dilute the remaining wash buffer (20×) after the erystallization is completely dissolved. Remaining wash buffer(20×) can be stored in 2-8°C.
3. Standerd preparntion: Standards are diluted to 5 ng/mL with sample dilution and then prepared by 2.5 times the dilution method.
4. Substrate Solution: Mix the chromo-developing solution A and chromo-developing solution B with equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution afher mixing has turmed blue, do not use it.

Assay Procedure

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8°C.
2. Preparation of the standard curve: dilute the Benzonase standard with sample dilution to 5ng/ml, then prepared by 2.5 times the dilution method.



3. Temperature breeding of samples: set up standard holes, blank holes and sample holes. l00µL of standard substances of diferent concentrations were added to the standard holes in order, and 100µL of sample dilution to the blank holes, and 100µL of samples to be tested were added to the sample holes, and then incubated at 37 temperature for 1h.
4. Washing board: discard the liquid in the hole and wash the board with 1× washing liquid for 3 times (250µ

L/hole). Pat dry residual liquid in sample hole. (Before discarding the liquid in the hole each time, if washing plate by hand, the liquid can be left for 1 min after adding and slightly shaken; If plate washing machinc is used, 5s can be slightly shaken after adding wash buffer)

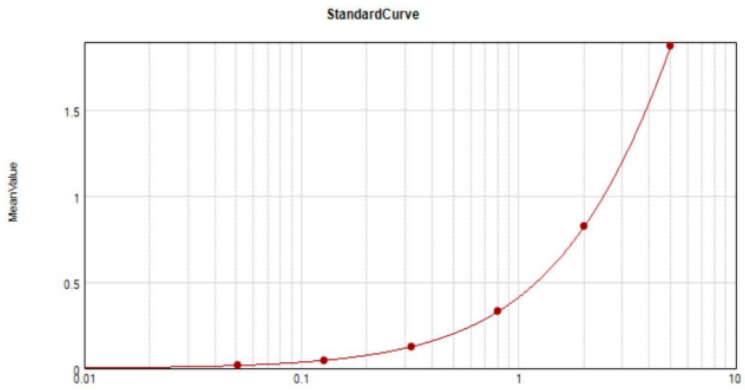
5. HRP-conjugate benzonase antibody incubate: Add 100μL of HRP-conjugate benzonase antibody each well, and then incubate at 37 for 1h.
6. Washing board: discard the liquid in the hole and wash the board with 1× washing liquid for 3 timcs (250μ L/hole). Pat dry residual liquid in sample hole.
7. Color development: the pre-prepared substrate solution was added to the enzyme label plate, mixed well (100μL/hole), and incubated in darkness for 15min.
8. Termination: add stop solution (100μL/hole)
9. Recording: The OD value at 450/630nm should be measured by placing the plate in the marker, and the reading should be completed within 20min.

Calculation

If samples generate values higher than the highest standard, dilute the sample and repeat the assay.The average OD value of each standard product minus the OD value of the blank hole as the correction value. The OD450nm value of the Benzonase standard was taken as the dependent variable Y, and the standard concentration was taken as the independent variable X to make the curve. We recommend to use four-parameter fitting equation: Y=((A-D)/(1+(x/C^B))+D. The absorbance value of the sample OD450nm was substituted into the formula to calculate the Benzonase content of the sample.

Typical Standard Curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Standard (ng/mL)	OD_{450}
5	2.063
2	0.914
0.8	0.398
0.32	0.177
0.128	0.113
0.0512	0.081
0	0.061

Detection Range

0.05 - 5 ng/mL

Precautions

1. All reagents of this kit are intended for professional use only.
2. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
4. Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
5. Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
6. Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
7. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as nonhazardous.
8. During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

   In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
9. Personal precautions, protective equipment and emergency procedures:

   Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

   Exposure controls/personal protection: Wear protective gloves of nitril rubber or natural latex.

Wear protective glasses. Used according to intended use no dangerous reactions known.

10. Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
11. For disposal of laboratory waste the national or regional legislation has to be observed.