BESTCHROM
博 格 隆

# BVH ELISA Kit

# Instruction for use

# Contents

## 1. Introduction

BVH ELISA Kit is a type of Sandwich ELISA kit, which is used for the detection of residual BVH ligand leakage in pharmaceutical products after using BVH Diamond resin in purification process.

Kit characteristics:

- High detection sensitivity, standard curve OD ratio of 0pg/mL and 160pg /mL is approximately 2 to 3 times.
- High precision, inter-and intra-plate variability is <10%.
- Acid-treatment for Sample pre-treatment, eliminate sample boiling and the subsequent centrifugation steps. Incubation step is carried out at the same time of antibody/antigen capture and antibody detection. Eliminate inconvenience of multiple plate washing. Simplify operation.

## 2. Detection Principle

BVH ELISA Kit can detect BVH residue from samples via Two-site Sandwich ELISA approach. First dilute BVH-containing sample with Sample Diluent (provided in the Kit). Add denaturing Buffer and mix to isolate BVH and sample antibody. Add denatured sample to and let it react with the polycolonal anti-BVH pre-coated ELISA Microplate. A second anti-BVH antibody labeled directly with horse radish peroxidase (HRP) enzyme is simultaneously reacted forming as sandwich complex of solid phase antibody-BVH-enzyme-linked antibody. After a wash step to remove any unbound reactants, the strips are then react with tetramethyl benzidine (TMB) substrate, producing a color change (from colorless to blue). It will finally turn to yellow after using Stop Solution. The more BVH ligands residue is in sample, the darker the color will be.The Detect optical density (OD) at 450nm and 650nm. OD shall be positively related to BVH contents in sample.

## 3. Kit Component

| S/N | Component | Pack size | Description |
|---|---|---|---|
| 1 | Anti-BVH pre-coated Microplate | 8 wells x12 strips | Dismantable, depend on practical requirement |
| 2 | BVH Standard | 8 vials×1mL | 0 ng/mL, 0.16 ng/mL, 0.31 ng/mL, 0.63 ng/mL, 1.25 ng/mL, 2.5 ng/mL, 5 ng/mL, 10ng/mL |
| 3 | Anti-BVH:HRP(100x) | 150μL/vial | Enzyme-linked antibody, use Anti-BVH: HRP Diluent get 1 x dilution |
| 4 | Anti-BVH:HRP Diluent | 12mL/bottle | Enzyme-linked antibody diluent |

| S/N | Component | Pack size | Description |
|---|---|---|---|
| 5 | Denaturing Buffer | 12mL/bottle | Denaturation buffer<br>-- Citrate buffer solution for denaturation treatment of sample |
| 6 | Sample Diluent | 25mL/bottle | Sample Diluent |
| 7 | Stop Solution | 12mL/bottle | Acid solution, corrosive<br>——Stop solution |
| 8 | TMB | 12mL/bottle | Solution containing 3,3',5,5' – tetramethylbenzidine and $H_2O_2$ |
| 9 | Wash Concentrate(10x) | 50mL/bottle | Use deionized water to get 1x dilution |
| 10 | Sample Treatment Plate | 96 well PCR plate | For denaturation treatment of sample |

## 4. Assay Protocol

- Kit preparation: bring all reagents to room temperature.
- Set up reagents:
  - Anti-BVH: HRP (100x): use Anti-BVH: HRP Diluent to get 1x dilution.
  - Wash Concentrate (10x): use deionized water to get 1x dilution.
- Sample preparation:
  - Standard: before using, mix with a vortex mixer, centrifuge sample.
  - Sample to be tested: Dilute with 1-fold Sample Diluent and set aside for use. It is recommended to get 10-fold or above dilution. Sample volume shall be no less than 100μL after dilution.
  - Spiked sample: Spiked sample recovery rate detection is an important criterion for the applicability and methodological validation of the experimental system. Volume of spiked sample shall be no less than 100μL.
- Experimental procedure:
  - Bring all reagents, components of kit to room temperature. Conduct all operation at room temperature. It is recommended to run sample in replicate for all wells (with sample).
  - Add sample:

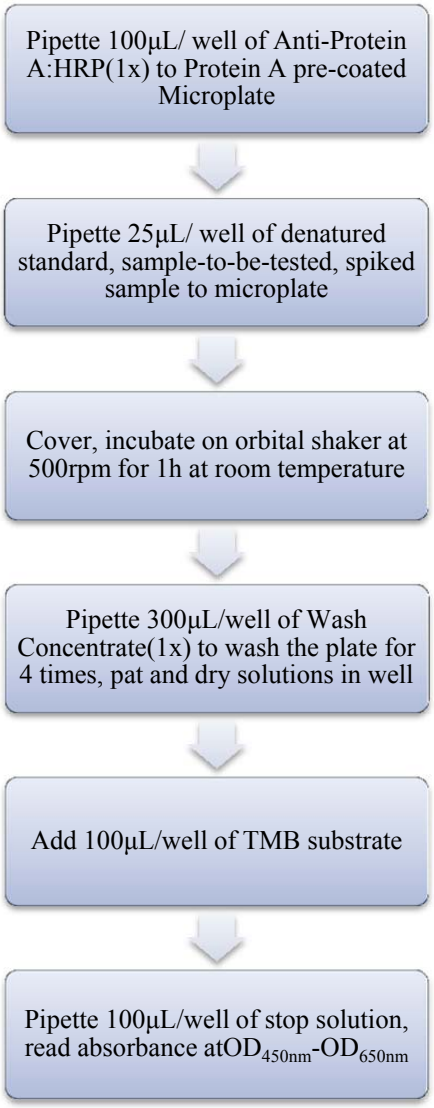
    1） Using Sample Dilute, dilute sample-to-be-tested and spiked sample to the right ratios. Add 50μL/ well of solution(the above-mentioned two solutions) to Sample Treatment Plate. Add 25μL of Denaturing Buffer to each well. Using a pipette to suck and blow, to mix solution well, repeat the process for at least 10 times (keep doing it at the right speed, avoid introducing any air). Let the treated sample stand still for 10 min and set aside for use.

    2） Pipette 100μL of Anti-Protein A: HRP (1x) to each well of polyclonal anti-BVG coated

microplate. Pipetter 25μL/well of denatured sample-to-be-tested, spiked sample and standard sample to microplate. Make sure run sample in replicate for each sample/standard sample (parallel). Incubate at 500rpm for 1hours.

- Wash: Shake the microplate to dry it. Pipette 300μL/ well of Wash Concentrate (1x),wash the microplate, repeat the washing process for 4 times.
- Coloring: pipette 100μL/ well of TMB substrate, incubate at room temperature in dark for 10min until the color of max concentration standard to dark blue.
- Ending: add 100 μL/well of Stop Solution (Color turns from blue to yellow).
- Read absorbance (OD) at 450nm and 650nm. Calculate the difference of OD between 450nm and 650 nm.

- Workflow of leached BVH detection

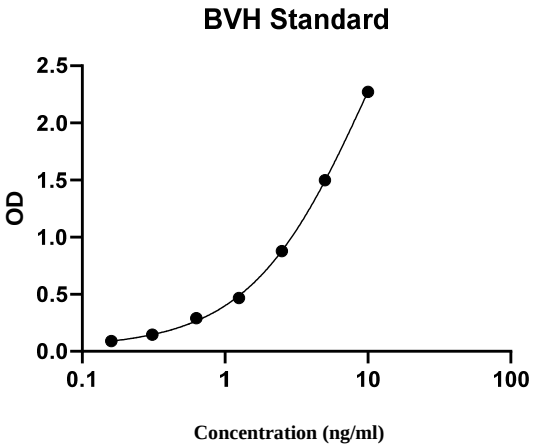


## 5. Data Analysis

- The calculation of OD: for each standard or sample, $OD=OD_{450nm}-OD_{650nm}$, OD should be the average value of two repetitive wells. Use microplate reader software or other softwares such as GraphPad Prism to analysis data. Using standard concentration as abscissa(X) and standard absorbance concentration as ordinate(Y), get standard curve. It is recommended to use four-parameter curve fitting equation. Calculate BVH content in sample and spiked sample using the fitted standard curve. Pay attention to the dilution factor.

➢ For standard curve of BVH Standard

| BVH Standard conc. (ng/mL) | Average OD |
|---|---|
| 10 | 2.271 |
| 5 | 1.499 |
| 2.5 | 0.878 |
| 1.25 | 0.468 |
| 0.63 | 0.290 |
| 0.31 | 0.147 |
| 0.16 | 0.091 |
| 0 | 0.027 |




## 6. Product Performance

- Spiked experiment and recovery validation: Evaluation of BVH Standard with the existence of BVH-affinity antibody. Since antibody has good affinity to BVH, mix 250ng/mL of BVH with 25mg/mL of antibody according to volume ratio of 1:1(ppm=10). Dilute the mixture with Sample Diluent (1x), do denaturalizing treatment to sample. Get measured concentration of sample according to standard curve of the kit. Calculate the expected percentage value by dividing the measured concentration by the expected concentration.
- Precision: intra and inter-assay precision.

➢ Intra-assay precision:

Conduct 16 repetitive detections to 3 BVH control samples via single experiment

| #of Tests | BVH Standard Concentration(pg/mL) | %CV |
|---|---|---|
| 16 | 8 | 4.8 |
| 16 | 3 | 6.7 |
| 16 | 0.5 | 6.4 |

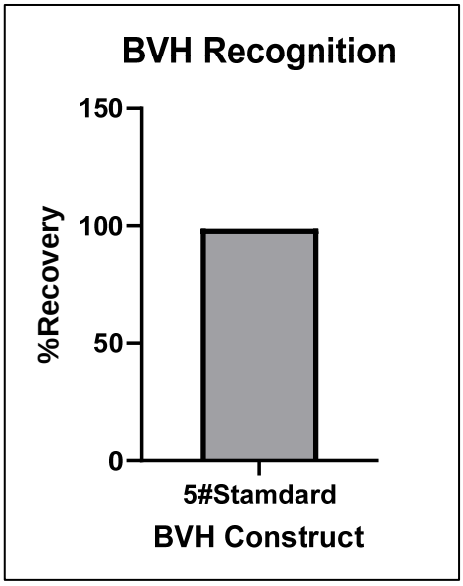
➢ Inter-assay precision:

Using 3 batches of Kits to detect the BVH-containing control sample

| #of Tests | BVH Standard Concentration(pg/mL) | %CV |
|---|---|---|
| 3 | 8 | 6.2 |
| 3 | 3 | 4.7 |
| 3 | 0.5 | 5.5 |

- Specificity: BVH ELISA Kit can specifically identify BVH Standard. The following picture shows evaluation of BVH antibody.

  Get measured concentration of sample according to standard curve of the kit. Calculate the expected percentage value (n=9) by dividing the measured concentration by the expected concentration.




## 7. Precautions

- Before using, all components of kit shall be restored to room temperature.
- HOOK effect:Within certain concentration range, concentration of analyte shows positive correlation to detection signal. However, when analyte concentration reaches to a certain level, signal will decrease despite of the increase of analyte concentration. This phenomenon gets its name “hook effect” for the hook shape of concentration/signal curve. When BVH concentration is excessively high, detection level can be lower than 10ng/mL. In that case, dilution of sample can usually solve the issue. Therefore, when detecting BVH leakage, it is recommended to conduct dilution linear evaluation to eliminate the impact of “Hook effect”.
- For recovery detection by adding BVH (with known content), accepted recovery range is usually from 80%-120%. Extreme pH or salinity might cause abnormal recovery. Under some conditions, high concentrated antibody will cause negative interference. In that case, please contact

Bestchrom Technical Support Team for help.

- Avoiding using pipette tip to touch the bottom of microplate, to prevent any damage to pre-coating.
- After washing the ELISA microplate, tap it to dry it. Make sure no strip is falling out.
- During the reaction, to minimize the solution evaporation from microplate, it is recommended to cover the ELISA microplate and Sample Treatment Plate using the covering membrane provided in Kit.
- Use the kit within its validity. When detecting BVH leakage, it is necessary to use the right standard. Please avoid using reagents from different batches.
- Detection results variation might be caused by various factors, including experimental staff operation, the use of pipette, plate washing method, reaction time, contaminant in bottom of ELISA microplate, or temperature.
- This kit is for in vitro research experiments only, not for clinical diagnosis.
- Other needed reagents(not provided by kit): deionized water, 1.5mL low-adsorption centrifuge tubes, high precision pipette & low adsorption pipette tip, tissue paper, microplate reader, microplate shaker(200-500rpm), software can be used for the fitting of four-parameter curve(e.g. GraphPad Prism).

## 8. Storage

Kit: Preserved at 2-8℃.

## 9. Ordering Information

| Product | Cat.No |
|---|---|
| BVH ELISA Kit | EK002 |
| Anti-BVH pre-coated Microplate | EK002-01 |
| BVH Standard | EK002-02 |
| Anti-BVH:HRP(100x) | EK002-03 |
| Anti-BVH:HRP Diluent | EK002-04 |
| Denaturing Buffer | EK002-05 |
| Sample Diluent | EK002-06 |
| Stop Solution | EK002-07 |
| TMB | EK002-08 |
| Wash Concentrate(10x) | EK002-09 |
| Sample Treatment Plate | EK002-10 |