Mouse Hexosaminidase B Beta (β-Hex) ELISA Kit

Cat. No.: EJ1512586 | Pack size: 48T / 96T | Storage: Store at 2-8 °C

Overview

β-Hexosaminidase (β-Hex), also known as hexosaminidase, is involved in the hydrolysis of terminal N-acetyl-D-hexosamine residues from N-acetyl-β-D-hexosamine residues.

This kit uses a double-antibody sandwich ELISA to detect mouse β-Hex in serum, plasma and tissue homogenate samples. The absorbance at 450 nm is used to calculate sample concentration.

Key Features

- Double-antibody sandwich ELISA for mouse β-Hex
- Detection range: 0.156 ng/mL - 10 ng/mL
- Sample types: plasma, serum and tissue homogenates
- Intra- and inter-assay CV values are both <10%
- Detection duration is approximately 3.5 hours, excluding equilibration and sample preparation

Product Information

Item	Description
Specifications & Purity	BioReagent, for enzyme immunoassay (ELISA)
Application	ELISA
Sample Type	Plasma, serum, tissue homogenates
Detection Range	0.156 ng/mL - 10 ng/mL
Method of Detection	ELISA; double-antibody sandwich method
Detected Species Object	Mouse
Specificity	Detects mouse β-Hex with no significant cross-reactivity to other homologs.
Detection Instrument / Wavelength	Microplate reader / 450 nm
Repeatability	Intra- and inter-assay CV <10%

Item	Description
Storage & Shipping	Store at 2-8 °C; shipped on wet ice; shelf life 6 months

Contents & Storage

Cat. No.	Components	48T	96T	Storage
EJ1512586A	Pre-coated Assay Plate	48 well	96 well	2-8 °C
EJ1512586B	Standard	1 tube	2 tubes	2-8 °C
EJ1512586C	Universal Diluent	1 x 20 mL	2 x 20 mL	2-8 °C
EJ1512586D	Biotin-antibody (100x)	60 µL	120 µL	2-8 °C
EJ1512586E	Streptavidin-HRP (100x)	60 µL	120 µL	2-8 °C
EJ1512586F	Wash Buffer (20x)	1 x 10 mL	2 x 10 mL	2-8 °C
EJ1512586G	TMB Substrate	5 mL	10 mL	2-8 °C
EJ1512586H	Stop Solution	3 mL	6 mL	2-8 °C
EJ1512586I	Plate Sealer	4 pieces	4 pieces	2-8 °C

Note: EJ1512586B, EJ1512586D, EJ1512586E and EJ1512586F shall be diluted according to the instructions.

Shelf life: Each component has a shelf life of 6 months under corresponding storage conditions.

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Microplate reader	450 nm	Measurement of OD value
High-precision pipettes and tips	0.5-10 µL, 5-50 µL, 20-200 µL, 200-1000 µL	Sample and reagent dispensing
37 °C incubator	Stable temperature control	Plate incubation
Distilled or deionized water	Freshly prepared	Preparation of 1x wash buffer

Item	Recommended Specification	Purpose
Microcentrifuge tubes	Clean, labeled EP tubes	Standard and sample dilution
Wash bottle or plate washer	Compatible with 96-well plates	Plate washing

Experimental Principle

This kit adopts a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Samples, standards, biotin-labeled detection antibody and HRP conjugate are sequentially added to microwells pre-coated with mouse β-Hex capture antibody.

After incubation and washing, TMB substrate is used for color development. TMB is catalyzed by HRP to blue and converted to yellow under acidic conditions. The color intensity is positively correlated with the concentration of mouse β-Hex in the sample. The absorbance (OD value) is measured at 450 nm and the sample concentration is calculated from the standard curve.

Sample Handling and Requirements

1. The kit detection range does not necessarily equal the analyte concentration range in samples. Estimate sample concentration from literature or a preliminary experiment; dilute or concentrate samples appropriately if needed.
2. If the sample type is not listed in this manual, run a preliminary experiment to verify detection validity.
3. Serum: allow whole blood collected in serum separation tubes to stand at room temperature for 2 hours or overnight at 2-8 °C, then centrifuge at 1000 x g for 20 minutes and collect the supernatant. Store at -20 °C or -80 °C if needed; avoid repeated freeze-thaw cycles.
4. Plasma: collect using EDTA or heparin as anticoagulant, centrifuge at 1000 x g for 15 minutes at 2-8 °C within 30 minutes of collection, and use the supernatant for testing. Store at -20 °C or -80 °C if needed; avoid repeated freeze-thaw cycles.
5. Tissue homogenate: rinse tissue with pre-chilled PBS (0.01 M, pH 7.4) to remove residual blood. Weigh and mince tissue, add PBS at about 1:9 (w/v), homogenize on ice, then further lyse by sonication or repeated freeze-thaw if needed. Centrifuge at 5000 x g for 5-10 minutes and use the supernatant.
6. Cell culture supernatant: centrifuge at 1000 x g for 20 minutes and use the supernatant for testing. Store at -20 °C or -80 °C if needed; avoid repeated freeze-thaw cycles.
7. Cell lysate: wash cells with pre-chilled PBS, collect by centrifugation at 1000 x g for 5 minutes, wash three times with PBS, resuspend in 150-200 µL PBS per 1 x 10^6 cells, lyse by freeze-

thaw or sonication, then centrifuge at 1500 x g for 10 minutes at 2-8 °C and use the supernatant.

8. Other biological samples: centrifuge at 1000 x g for 20 minutes and use the supernatant for testing.
9. Samples should be clear and transparent. Remove suspended matter by centrifugation. Hemolyzed samples should not be used because hemolysis interferes with results.
10. If testing within 1 week, store samples at 4 °C. For delayed testing, aliquot and store at -20 °C for up to 1 month or -80 °C for up to 6 months; avoid repeated freeze-thaw cycles.

Sample Dilution Protocol

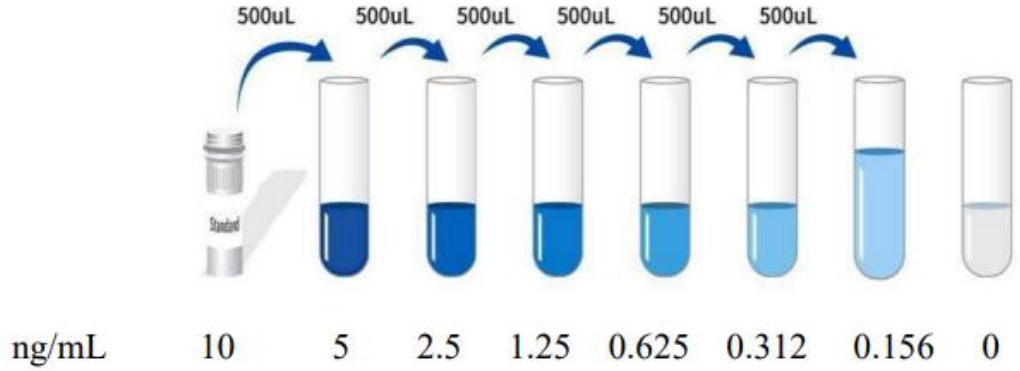
Estimate the sample concentration range in advance. If dilution is required, follow the protocol below. In each step, use at least 3 µL for pipetting and do not exceed 100-fold dilution per step. Mix thoroughly after each dilution and avoid bubbles.

1. 100-fold dilution: add 5 µL sample to 495 µL general diluent.
2. 1000-fold dilution: add 5 µL sample to 95 µL general diluent (20-fold), then add 5 µL of the 20-fold dilution to 245 µL general diluent (50-fold); total dilution = 1000-fold.
3. 100,000-fold dilution: add 5 µL sample to 195 µL general diluent (40-fold), then add 5 µL of the 40-fold dilution to 245 µL general diluent (50-fold), then add 5 µL of the 2000-fold dilution to 245 µL general diluent (50-fold); total dilution = 100,000-fold.

Pre-Test Preparation

1. Remove the kit from the refrigerator 10 minutes in advance and equilibrate it to room temperature.
2. Prepare standard gradient working solution: add 1 mL universal diluent to the lyophilized standard, stand for 15 minutes until completely dissolved, and mix gently. The top standard concentration is 10 ng/mL. Prepare serial dilutions to 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0 ng/mL.

Serial dilution method: place 7 EP tubes, each containing 500 µL universal diluent. Transfer 500 µL of the top standard working solution to the first tube and mix; repeat sequential transfer and mixing. The last tube is used as the blank well and no liquid is transferred from the penultimate tube.



3. Prepare biotin-antibody working solution: centrifuge the 100x concentrated biotin-antibody at 1000 x g for 1 minute 15 minutes before use. Dilute to 1x with universal diluent (for example, 10 µL concentrate + 990 µL diluent). Prepare freshly before use.
4. Prepare enzyme conjugate working solution: centrifuge the 100x concentrated enzyme conjugate at 1000 x g for 1 minute 15 minutes before use. Dilute to 1x with general diluent. Prepare freshly before use.
5. Prepare 1x washing solution: add 10 mL of 20x washing solution to 190 mL distilled water. If crystals are present in concentrated wash buffer, allow it to reach room temperature and dissolve completely before preparation.

Procedure

1. Remove the required strips from the aluminum foil bag after equilibration at room temperature for 10 minutes. Seal the remaining strips in a resealable bag and return to 4 °C.
2. Sample addition: add 100 µL of samples or standards of different concentrations to the corresponding wells. Add 100 µL universal diluent to blank wells. Cover with a plate sealer and incubate at 37 °C for 60 minutes. Duplicate wells are recommended for all samples and standards.
3. Add biotinylated detection antibody: discard liquid without washing, then add 100 µL biotinylated detection antibody working solution to each well. Cover and incubate at 37 °C for 60 minutes.
4. Wash plate: discard liquid, add 300 µL 1x washing buffer to each well, stand for 1 minute, discard wash buffer and pat dry on absorbent paper. Repeat washing 3 times or use a plate washer.
5. Add enzyme conjugate working solution: add 100 µL to each well. Cover and incubate at 37 °C for 30 minutes.
6. Wash plate 5 times as described above.
7. Add substrate: add 90 µL TMB substrate to each well. Cover and incubate at 37 °C in the dark for 15 minutes.

8. Add stop solution: add 50 µL stop solution to each well and immediately measure OD at 450 nm.

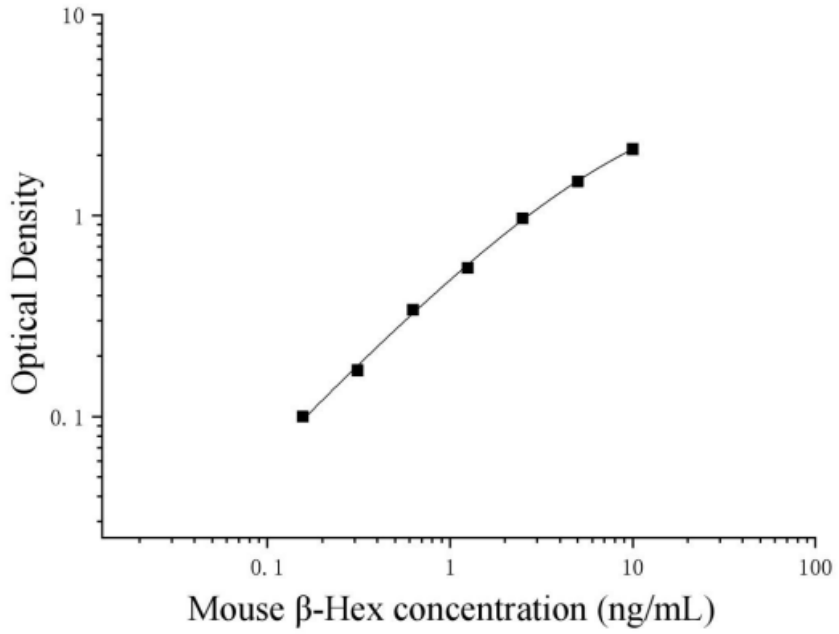
Calculation of Experimental Results

1. Calculate the average OD value of duplicate standards and samples, then subtract the blank OD value to obtain the corrected OD value. Plot a standard curve using a four-parameter logistic function, with concentration on the x-axis and OD value on the y-axis.
2. If the sample OD value exceeds the upper limit of the standard curve, dilute the sample appropriately and re-test. Multiply the final concentration by the dilution factor.

Typical Data and Reference Curve

The following data and curve are for reference only. Users must establish a standard curve based on each experiment.

Concentration (ng/mL)	10	5	2.5	1.25	0.625	0.312	0.156	0
OD value	2.39	1.67	1.17	0.71	0.52	0.33	0.27	0.1
Corrected OD value	2.29	1.57	1.07	0.61	0.42	0.23	0.17	-



Kit Performance

1. Repeatability: Intra-assay CV< 10%; inter-assay CV< 10%.
2. Recovery rate: Mouse β-Hex at three different concentration levels was spiked into selected healthy mouse serum, plasma and cell culture supernatant, and the recovery rate was calculated.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	84-101	96
Plasma (n=8)	92-105	102
Cell culture supernatant (n=8)	96-108	105

3. Linear dilution: High-concentration mouse β-Hex was spiked into 4 selected healthy mouse serum, plasma and cell culture supernatant samples respectively. Serial dilutions were performed within the dynamic range of the standard curve to evaluate linearity.

Dilution Ratio	Recovery (%)	Serum	Plasma
1: 2	Range	84-95	88-96
1: 2	Average recovery	91	93
1: 4	Range	89-103	87-108
1: 4	Average recovery	94	98

Safety & Precautions

1. Bring all reagents to room temperature (20-25 °C) before use and store reagents at 2-8 °C immediately after use.
2. Strictly follow the specified incubation time and temperature. Inadequate incubation or washing may cause inaccurate results.
3. Do not allow microwells to dry during the assay. Drain wells thoroughly before adding substrate.
4. Clean residual liquid and fingerprints from the well bottoms before reading optical density.
5. Use colorless TMB substrate only. Do not use substrate that has turned blue before use.
6. Avoid cross-contamination between reagents and samples. Do not mix kit components from different catalog numbers or lots.

7. Protect reagents and plates from strong light during storage and incubation.
8. Handle all biological samples according to established laboratory biosafety procedures.

Quality Control

QC Item	Method	Acceptable Range
Standard curve	Run standards in duplicate	Curve covers specified detection range and blank-corrected OD values are suitable for interpolation.
Precision	Duplicate measurements	Intra- and inter-assay CV <10%.
Kit integrity	Receipt and storage inspection	Components complete; stored at 2-8 °C; expiry date and lot number legible.

Troubleshooting

Issue	Possible Causes	Corrective Action
Poor standard curve linearity	Incorrect standard dilution	Dissolve and serially dilute standards exactly as described. Mix thoroughly and avoid bubbles.
Poor standard curve linearity	Inaccurate pipetting	Calibrate pipettes regularly and check tip sealing. Run standards in duplicate.
Poor standard curve linearity	Evaporation of reaction solution	Seal the plate with film during incubation.
Poor standard curve linearity	Inadequate washing	Use sufficient wash buffer volume and repeat the recommended wash times.
Weak or no color development	Insufficient incubation time or incorrect temperature	Ensure full incubation at 37 °C for each specified step.
Weak or no color development	Incorrect reagent dilution	Verify dilution of biotin-antibody, HRP conjugate and wash buffer.
Weak or no color development	Inactivated enzyme conjugate	Check for color development with conjugate and substrate; replace reagents if needed.

Issue	Possible Causes	Corrective Action
Low OD values	Incorrect reader wavelength or delayed reading	Read at 450 nm immediately after adding stop solution.
OD value outside standard range	Sample concentration too high or too low	Dilute or concentrate the sample based on a preliminary experiment and re-test.
High background	Contaminated substrate or excessive development time	Replace substrate if needed and control color development time.
High background	Inadequate washing	Wash thoroughly and pat dry on absorbent paper after each wash sequence.

Recommended Applications

ELISA detection of mouse β-Hex · Serum samples · Plasma samples · Tissue homogenates · Double-antibody sandwich immunoassay research

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