



User's Manual

Mouse Vitamin B6 ELISA Kit

REF DEIA-BJ2629

 96T



RUO

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

Principles of Testing

This assay is based on the competition ELISA principle. Each well of the supplied microtiter plate has been pre-coated with an antigen-specific capture antibody. Standards or samples are added to each well as well as a fixed quantity of Horseradish Peroxidase (HRP)-conjugated target antigen. The free antigen in the sample competes with the HRP-conjugated antigen for binding to the capture antibody. Unbound antigen is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration. In contrast to typical Sandwich ELISA assays in competition assay the greater the amount of antigen in the sample the lower the color development and optical density reading.

Reagents And Materials Provided

1. Coated 96-well Strip Plate 1
2. Standard A (0 nmol/l) 1 vial
3. Standard B (1.0 nmol/l) 1 vial
4. Standard C (2.5 nmol/l) 1 vial
5. Standard D (5.0 nmol/l) 1 vial
6. Standard E (10 nmol/l) 1 vial
7. Standard F (25 nmol/l) 1 vial
8. Balance Solution 1 vial x 3 mL
9. HRP-Conjugate 1 vial x 6 mL
10. Wash Buffer (100x) 1 vial x 10 mL
11. Substrate A 1 vial x 6 mL
12. Substrate B 1 vial x 6 mL
13. Stop Solution 1 vial x 6 mL
14. Adhesive Plate Sealers 4
15. Instruction Manual 1

Materials Required But Not Supplied

1. Microplate reader with 450 nm wavelength filter
2. High-precision pipette and sterile pipette tips
3. Eppendorf tubes
4. 37°C incubator
5. Deionized or distilled water
6. Absorbent paper

Storage

The unopened kit can be stored at 2°C to 8°C through the expiration date. Once opened the kit can be stored at 2°C to 8°C for 1 month. Unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air.

Specimen Collection And Preparation

This assay is recommended for use with Mouse Plasma and Serum. Use with other sample types is not supported. The following are a collection of sample collection protocols for your reference.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 × g at 2°C to 8°C within 30 minutes of collection. Collect the supernatant for assaying. Store undiluted samples at -20°C or below. Avoid repeated freeze-thaw cycles

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at approximately 1,000 × g. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Sample Collection Notes

1. CD recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18°C to 25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assaying. If the sample concentration is not within the range of the standard curve users must determine the optimal sample dilutions for their particular experiments.
8. CD is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples use with the kit.

SAMPLE PREPARATION

The resulting Optical Density (OD) values of your sample must fall within the OD values of the standard curve in order for the calculated antigen concentration to be accurate. In many cases samples will need to be diluted in order to lower the antigen concentration to sufficient levels. Information about antigen concentrations within various sample types may be available from the published literature; however it is often necessary to run a dilution series of each sample type. The following will prepare sufficient volumes to run the Sample dilution series in triplicate. In the case of small volume samples, a preliminary step dilution, such as 1:5 or 1:10, can be made using PBS (0.02 mol/L pH 7.0-7.2) as the diluent. *The final dilution should always be made using the same buffer that is used to dilute the Standards, and/or generate the Standard Curve. Running duplicate or triplicate wells for each sample is recommended.

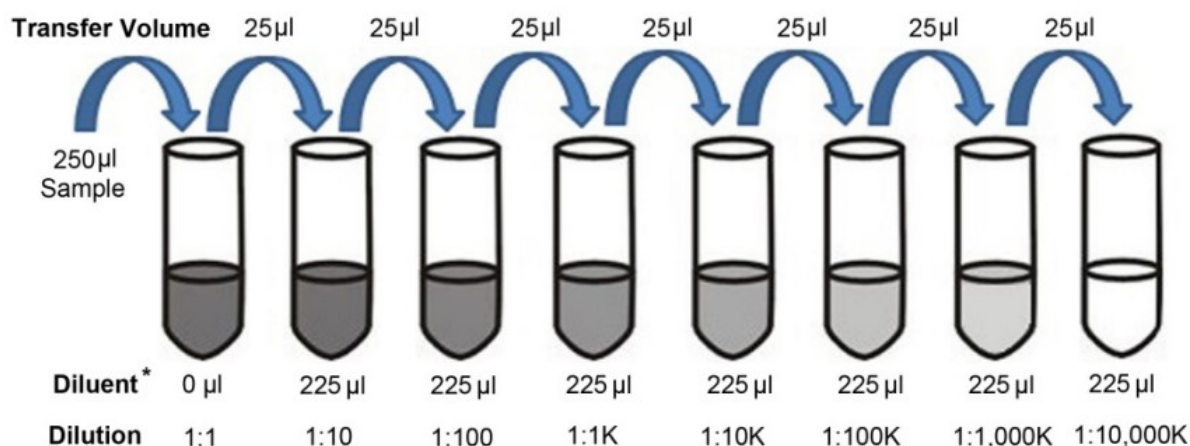


Plate Preparation

The following is an example of how to layout a study. A dilution series of the positive control Standard should be run in duplicate or triplicate with the last well in each series being the negative control blank. Samples should also be run in duplicate or triplicate. Unknown samples should be run as a dilution series in order to identify the optimal dilution that produces an OD reading within the OD range of the positive control Standard dilution series.

Example 1: Standard Curve and dilution series of an unknown sample.

	1	2	3	4	...
A	Standard A	Standard A	Sample (1:1)	Sample (1:1)	...
B	Standard B	Standard B	Sample (1:10)	Sample (1:10)	...
C	Standard C	Standard C	Sample (1:100)	Sample (1:100)	...
D	Standard D	Standard D	Sample (1:1k)	Sample (1:1k)	...
E	Standard E	Standard E	Sample (1:10k)	Sample (1:10k)	...
F	Standard F	Standard F	Sample (1:100k)	Sample (1:100k)	...
G	Blank	Blank	Sample (1:1,000k)	Sample (1:1,000k)	...
H	Blank	Blank	Sample (1:10,000k)	Sample (1:10,000k)	...

Example 2: Standard Curve and samples run in duplicate

	1	2	3	4	...
A	Standard A	Standard A	Sample A	Sample E	...
B	Standard B	Standard B	Sample A	Sample E	...
C	Standard C	Standard C	Sample B	Sample F	...
D	Standard D	Standard D	Sample B	Sample F	...
E	Standard E	Standard E	Sample C	Sample G	...
F	Standard F	Standard F	Sample C	Sample G	...
G	Blank	Blank	Sample D	Sample H	...
H	Blank	Blank	Sample D	Sample H	...

Reagent Preparation

Bring all reagents to room temperature (18°C to 25°C) before use.

1x Wash Buffer: If crystals have formed in the concentrate warm to room temperature and mix gently until crystals have completely dissolved. Prepare 1,000 mL of Working Wash Buffer by diluting the

supplied 10 mL of 100x Wash Buffer Concentrate with 990 mL of deionized or distilled water. Wash Buffer can be stored at 4°C once prepared. All other reagents are ready-to-use.

REAGENT PREPARATION NOTES

1. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
2. Reagents may adhere to the tube wall or cap during transport centrifuge tubes briefly before opening.
3. All solutions should be gently mixed prior to use.
4. To minimize imprecision caused by pipetting ensure that pipettes are calibrated. Pipetting volumes of less than 10 µL is not recommended.
5. Substrate Solution is easily contaminated so sterility precautions should be taken. Substrate Solution should also be protected it from light.
6. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
7. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

STANDARDS PREPARATION

The Standards supplied with this kit are ready-to-use and no preparation is necessary.

Assay Procedure

Bring all reagents and samples to room temperature without additional heating and mix thoroughly by gently swirling before pipetting (avoid foaming). Prepare all reagents, working standards, and samples as directed in the previous sections.

1. Add 100 µL of PBS (0.02 mol/L pH 7.0-7.2) to a Blank well.
2. Add 10 µL of Balance Solution to each 100 µL sample of cell culture supernatant or tissue homogenate if applicable, and mix well. Do NOT add to plasma or serum samples, standards, or the Blank.
3. Add 100 µL of Standard or Sample per well.

4. Add 50 μ L of 1x HRP-conjugate to each well (excluding the Blank well), gently agitate to ensure thorough mixing, cover with a plate sealer, and incubate for 1 hour at 37°C.
5. Aspirate the liquid from each well and wash five times. Wash by adding approximately 350 μ L of 1x Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser, or automated washer. Allow each wash to sit for 15 seconds before completely aspirating. After the last wash, aspirate to remove any remaining Wash Buffer then invert the plate and tap against clean absorbent paper.
6. Add 50 μ L of Substrate A and 50 μ L of Substrate B to each well (including the Blank), gently agitate to ensure thorough mixing, and incubate in the dark for 15-20 minutes at 37 °C.
7. Add 50 μ L of Stop Solution to each well. The blue color will change to yellow immediately. If color change does not appear uniform gently tap the plate to ensure thorough mixing. The Stop Solution should be added to wells in the same order and timing as was the substrate solutions.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm.

ASSAY PROCEDURE NOTES

1. **ELISA Plate:** Keep appropriate numbers of strips for one experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant and stored at 4°C.
2. **Solutions:** To avoid cross-contamination change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Applying Solutions:** All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. **Assay Timing:** The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells which will significantly affect the experimental accuracy and repeatability. For each step in the procedure total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. **Incubation:** To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. **Washing:** Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
7. **Controlling Substrate Reaction Time:** After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance reading.
8. **Reading:** The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
9. **Reaction Time Control:** Control reaction time should be strictly followed as outlined.
10. **Stop Solution:** The Stop Solution contains an acid therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
11. **Mixing:** During incubation times the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable results.
12. Kits from different batches may be a little different in detection range, sensitivity, and color developing time.

Please perform the experiment exactly according to the supplied instructions.

13. Due to inter- and intra-assay variability it is recommended that appropriate carry-over controls be included between assays.
14. Prior to running valuable samples CD recommends that the user run a preliminary experiment using the supplied controls in order to validate the assay.
15. To minimize external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, and incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
16. The kit should not be used beyond the expiration date on the kit label.

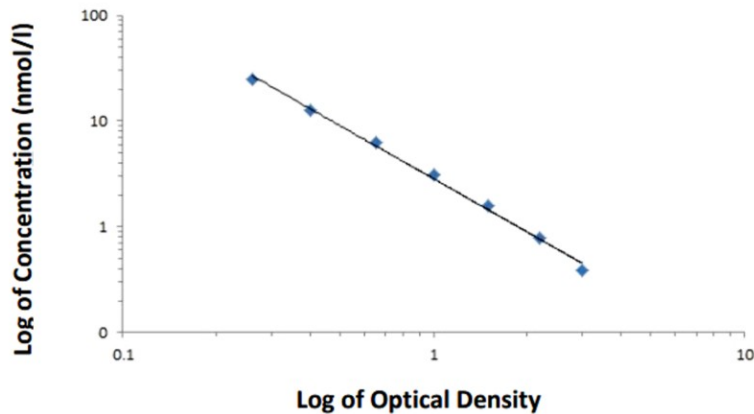
ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards.
2. Add 100 µL of PBS as a Blank well.
3. Add 10 µL of Balance Solution to each 100 µL sample of cell culture supernatant or tissue homogenate sample if applicable.
4. Add 100 µL of Standard or Sample to each well.
5. Add 50 µL of 1× HRP-conjugate to each well (excluding the blank) and incubate for 1 hour at 37°C.
6. Aspirate and wash five times.
7. Add 50 µL of Substrate A and 50 µL Substrate B to each well and incubate for 15-20 minutes at 37°C.
8. Add 50 µL of Stop Solution.
9. Read immediately at 450 nm.

Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average optical density of blank. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. The use of a commercial software program such as CurveExpert is recommended for performing this calculation. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data: The following standard curve is an example only and should not be used to calculate results for tested samples. A new standard curve must be generated for each set of samples tested.



Precision

Intra-Assay CV (< 10%)

Inter-Assay CV (< 10%)

Detection Range

1–25 nmol/L

Detection Limit

0.1 nmol/L

Specificity

This kit is for the detection of Mouse Vitamin B6. No significant cross-reactivity or interference between Vitamin B6 and analogs was observed. This claim is limited by existing techniques therefore crossreactivity may exist with untested analogs.

Precautions

This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.