Bovine NADP ELISA Kit

For the quantitative in vitro determination of Bovine nicotinamide adenine dinucleotide phosphate concentrations in

serum - plasma - tissue homogenates - other biological fluids

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

This package insert must be read in its entirety before using this product.

ELISA

ENZYME LINKED IMMUNOSORBENT ASSAY

Address: Kenneth G. Howell , 748 Nickel Road , San Francisco(415) , CA 94104
Telephone: 0014159141567

INTENDED USE AND TEST PRINCIPLE

This NADP ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of NADP in the sample, this NADP ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus NADP concentration. The concentration of NADP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

SAMPLE COLLECTION AND STORAGES

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 ℃ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at

1000×g. Remove particulates and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Note: The samples shoule be centrifugated dequately and no hemolysis or granule was allowed.

SAMPLE PREPARATION

1. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Sample should be diluted by 0.01mol/L PBS(PH=7.0-7.2).

3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.

5. Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

6. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.

7. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 ℃ incubator
2. Standard microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes, disposable pipette tips and Absorbent paper

4. Distilled or deionized water

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Name	96 determinations	48 determinations
MICROTITER PLATE	12*8strips	12*4strips
STANDARD（6 vial）	0.3ml/vial	0.3ml/vial
SAMPLE DILUENT	6.0ml	3.0ml
ENZYME CONJUGATE	10.0ml	5.0ml
WASH SOLUTION	25ml	15ml
SUBSTRATE A	6.0ml	3.0ml
SUBSTRATE B	6.0ml	3.0ml
STOP SOLUTION	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

Note:

1. Standard concentration was followed by: 32, 16, 8, 4, 2, 1 ng/mL.
2. If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.

8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

9. All samples should be disposed of in a manner that will inactivate viruses.

10. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

11. Substrate Solution is easily contaminated. If bluish prior to use, do not use.

12. Substrate B contain 20% acetone, keep this reagent away from sources of heat or flame.

13. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

REAGENT PREPARATION AND STORAGE

Wash Solution (1X) - Dilute 1 volume of Wash solution (20X) with 19 volumes of deionized or distilled water. Wash Solution is stable for 1 month at 2-8°C.

ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microtiter plate.

2. Add 50µl of Standard or Sample to the appropriate wells. Blank well doesn't add anyting.

3. Add 100µl of Enzymeconjugate to standard wells and sample wells except the blank well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

4. Wash the Microtiter Plate 4 times.

Manual Washing - Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper

or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µL/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5. Add Substrate A 50µl and Substrate B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**

6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (X) axis versus the corresponding concentration on the horizontal (Y) axis.

2. First, calculate the mean O.D. value for each standard and sample. All O.D. Values are subtracted by the mean value of the balnk well before result interpretation. Construct the standard curve using graph paper or statistical software.

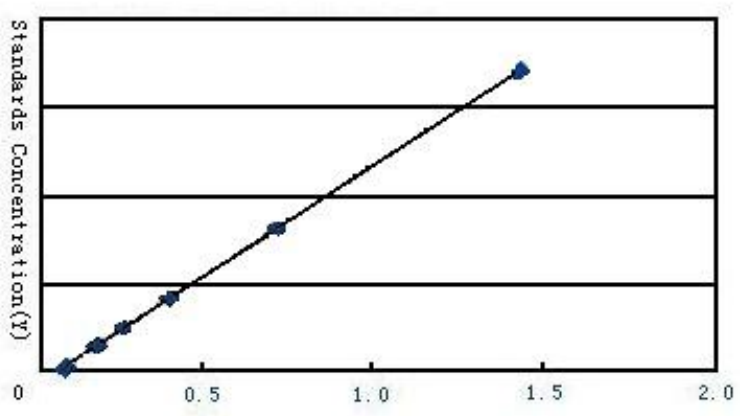
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.

4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

5. Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%.

6. Assay range: 1 ng/mL – 32 ng/mL.

7. Sensitivity: The minimum detectable dose of Bovine NADP is typically less than 0.1 ng/mL.
8. Cross-reactivity: This assay recognizes recombinant and natural Bovine NADP. No significant cross-reactivity or interference was observed.
9. Storage: 2-8℃ (Use frequently); six months (-20℃)。
10. Standard curve



IMPORTANT NOTE

1. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
2. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
3. 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 instruction attached in kit while electronic ones from our website is only for information.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

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TROUBLE SHOOTING

If the results are not good, please take pictures, keep the used plate and remaining reagents. Then contact our technical department. Meanwhile, you could refer to the following materials.

Problem	Possible Source	Correction Action
Poor Standard Curve	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and Add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate & substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Use new sample and repeat assay

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