



2,3-DPG ELISA Kit

Catalog Number KA1177

96 assays

Version: 14

Intended for research use only

www.abnova.com

Table of Contents

Introduction	3
Intended Use	3
Principle of the Assay	3
General Information	4
Materials Supplied	4
Storage Instruction	4
Materials Required but Not Supplied	4
Precautions for Use	4
Assay Protocol	6
Reagent Preparation	6
Sample Preparation	6
Assay Procedure	7
Data Analysis	9
Calculation of Results	9
Performance Characteristics	9
Resources	10
Plate Layout	10

Introduction

Intended Use

This immunoassay kit allows for the in vitro quantitative determination of general 2,3-Bisphosphoglycerate concentrations in serum, plasma, tissue homogenates, cell culture supernates, and other biological fluids.

Principle of the Assay

The ELISA is based on the competitive binding enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to 2,3-Bisphosphoglycerate. During the reaction, 2,3-Bisphosphoglycerate in the sample or standard competes with a fixed amount of biotin-labeled 2,3-Bisphosphoglycerate for sites on a pre-coated Monoclonal antibody specific to 2,3-Bisphosphoglycerate. Excess conjugated and unbound sample or standard are washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The concentration of 2,3-Bisphosphoglycerate in the samples is then determined by comparing the O.D. of the samples to the standard curve.

General Information

Materials Supplied

List of component

Component	Amount
Assay Plate	96 wells
Standard	2 vials
Sample Diluent	20 mL
Assay Diluent A	10 mL
Assay Diluent B	10 mL
Detection Reagent A	60 µL
Detection Reagent B	120 µL
Wash Buffer (25 x concentrate)	30 mL
Substrate	10 mL
Stop Solution	10 mL
Plate sealer for 96 wells	5 slices

Storage Instruction

The Assay Plate, Standard, Detection Reagent A and Detection Reagent B should be stored at -20°C upon being received. After receiving the kit, Substrate should be always stored at 4°C. Other reagents are kept according to the labels on vials. But for long term storage, please keep the whole kit at -20°C (Except the substrate). The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

Materials Required but Not Supplied

- ✓ Microplate reader.
- ✓ Pipettes and pipette tips
- ✓ EP tube
- ✓ Deionized or distilled water.

Precautions for Use

- ✓ Limitation of the procedure
- Abnova is not responsible for the samples consumed during the assay. The user should calculate the

possible amount of the samples used in the whole set. Please reserve sufficient samples in advance.

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with the Sample Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

✓ **Precaution**

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

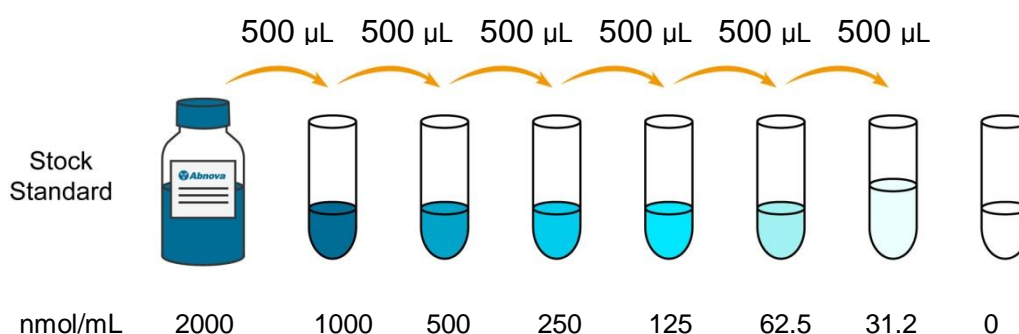
✓ **Important Note**

- *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.*
- *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.*
- *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.*
- *There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results.*
- *Do not remove microtiter plate from the storage bag until needed.*
- *A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.*
- *Use fresh disposable pipette tips for each transfer to avoid contamination.*
- *Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.*
- *Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.*
- *Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.*
- *Kits from different manufacturers for the same item might produce different results, since we haven't compared our products with other manufacturers.*
- *Valid period: six months.*

Assay Protocol

Reagent Preparation

- ✓ Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.
- ✓ Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 2000 nmol/mL. Allow the standard to sit for about 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the highest standard (2000 nmol/mL). The Sample Diluent serves as the zero standard (0 ng/mL).



- ✓ Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A or B (1:100), respectively.

Sample Preparation

- ✓ Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.
- ✓ Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- ✓ Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1 x PBS to remove excess blood, homogenized in 20 mL of 1 x PBS and stored overnight at ≤ -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at ≤ -20°C.

- ✓ Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note:

- *Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must store at -20°C (1 month) or -80°C (2 months) to avoid loss of bioactivity and contamination.*
- *Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA result due to the impacts of certain chemicals.*
- *Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit*
- *Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.*
- *When performing the assay slowly bring samples into room temperature.*

Assay Procedure

Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37°C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4°C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. 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.

1. Add 50 µL of Standard, Blank, or Sample per well.
2. Immediately add 50 µL of Detection Reagent A working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.
3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher and let it sit for 1~2 minutes. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of Detection Reagent B to each well. Cover with a new Plate sealer. Incubate for 45 minutes at 37°C.
5. Repeat the aspiration/wash process for five times as conducted in step 3.
6. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15-30 minutes at 37°C. Protect from light.
7. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Note:

- ✓ *Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.*
- ✓ *Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards Detection Reagent A and B can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 μ L for once pipetting.*
- ✓ *To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.*
- ✓ *For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.*
- ✓ *To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.*
- ✓ *The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.*
- ✓ *Duplication of all standards and specimens, although not required, is recommended.*
- ✓ *Substrate Solution is easily contaminated. Please protect it from light.*

Data Analysis

Calculation of Results

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. 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 2,3-Bisphosphoglycerate concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 1.3. 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.

Performance Characteristics

✓ Specificity

This assay recognizes recombinant and natural General 2,3-Bisphosphoglycerate. No significant cross-reactivity or interference was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between general 2,3-Bisphosphoglycerate and all the analogues, therefore, cross reaction may still exist.

✓ Detection Range

31.2-2000 nmol/mL

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H