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ProductInformation

Biliverdin Reductase Assay Kit

Catalog Number **CS1100**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Biliverdin reductase (BVR) catalyzes the transformation of the blue-green pigment biliverdin IX to the yellow-orange bile pigment, bilirubin IX.[1] A major feature of this enzyme is that it is the only enzyme shown to have two distinct cofactor dependent pH optima. In the acidic range of pH 6.0–6.7, NADH is utilized; whereas, in the alkaline range of pH 8.5–8.7, NADPH is used.[2] Biliverdin reductase is also known to contain a domain that acts as a serine/threonine/tyrosine kinase that belongs to the insulin receptor substrate family. Whereas, most tyrosine kinase activity is membrane bound, BVR is a soluble protein.[3]

Biliverdin reductase is also considered as a major physiologic cytoprotectant in the cell and may have other protective properties for diseased states of the cell. It has been shown to suppress experimental autoimmune encephalomyelitis in rats.[4] Depletion of the enzyme in the cell leads to accumulation of cellular oxidants and to augmented cell death.[5]

This kit contains all the reagents necessary for activity detection of biliverdin reductase. The formation of bilirubin from biliverdin is followed by a colorimetric reaction at 450 nm, in an NADPH dependent reaction, at pH 8.5 at 37 °C.

The kit was tested on tissue extracts prepared from rat liver, brain, heart, spleen, lung, skeletal muscle, and kidney. It was also tested on HEK-293T, BAEC, HepG2, COS, Jurkat, Balb/3T3, CHO, and U937 cell lines.

Components

The kit is sufficient for 200 assays of 200 µl in 96 well plates.

Assay Buffer 50 ml
Catalog Number A6356

Substrate Solution 250 µl
Catalog Number S7572

Biliverdin Reductase Positive Control 1 vial
Catalog Number B7186

NADPH 100 mg
(β-Nicotinamide adenine dinucleotide 2′-phosphate, reduced tetrasodium salt hydrate)
Catalog Number N6505

Equipment and reagents needed but not supplied

- 96 well plates (Catalog Number CLS3596 or equivalent)
- Plate reader with thermostatic control
  or
  Spectrophotometer with thermostatic control (optional)
- 160 µl cuvette (Catalog Number C9667, optional)
- Constant temperature bath
- Dulbecco`s Phosphate Buffered Saline (PBS) (Catalog Number D8537, optional)
- Trypsin-EDTA solution (Catalog Number T4049, optional)
- Extraction buffers – as required, see Sample Preparation

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Use ultrapure water (17 MΩ·cm or equivalent) for the preparation of reagents and throughout the procedure.

Positive Control Solution – Add 0.8 ml of ultrapure water to the vial of the Biliverdin Reductase Positive Control (Catalog number B7186). Mix by inversion. The Positive Control Solution may be frozen in working aliquots at –20 °C for up to 6 months.

NADPH Solution – Dissolve 4.6 mg of NADPH (Catalog number N6505) in 1 ml of Assay Buffer (Catalog Number A6356). Store at 2–8 °C. 1 ml of NADPH Solution is sufficient for at least 200 reactions; however, this solution should be freshly prepared for each experiment. Discard it after 5 hours. Keep the remainder of the NADPH powder at –20 °C.

Working Solution – Add 27 µl of NADPH Solution and 5 µl of the Substrate Solution (Catalog Number S7572) to 970 µl of Assay Buffer. Mix by inversion and warm at 37 °C for 5 minutes before use. Prepare the volume of Working Solution required for the experiment immediately before use.

Sample Preparation for Cell and Tissue Extracts

A. Cell Extract Preparation

The procedure is for cell extract preparations from 1–6 × 10^7 cells (in suspension or adherent cells) grown in a 150 cm^2 tissue culture flask.

1. Collect the cells from the flasks.
   - For cells in suspension centrifuge at 600 × *g* for 5 minutes.
   - For adherent cell lines - remove the cells from the flask with Trypsin/EDTA solution. Add 20 ml of growth medium (to neutralize the trypsin) and then centrifuge at 600 × *g* for 5 minutes.
2. Discard the supernatant.
3. Wash the cells with PBS and centrifuge at 600 × *g* for 5 minutes.
4. Discard the supernatant, suspend the pellet in 1 ml of PBS, and transfer to microcentrifuge tubes.
5. Centrifuge at 600 × *g* for 5 minutes. Discard the supernatant. For 1–6 × 10^7 cells, the packed cell volume should be 60-100 µl.
6. An Extraction Buffer of 25 mM HEPES, pH 7.4, with 1 mM EDTA is recommended. To the cell pellet add 150 µl of Extraction Buffer per ~65 µl of packed cell volume (for a different packed cell volume adjust the Extraction Buffer volume accordingly). Vortex to mix.
7. Break the cells with 15 strokes of a 2 ml Dounce homogenizer.
8. Centrifuge for 20 minutes at 16,000 × *g*.
9. Keep the supernatant liquid.
10. Re-extract the pellet with approximately half the Extraction Buffer volume (step 6) in a similar manner (step 7) and centrifuge again.
11. Pool the supernatants.

B. Tissue Extract Preparation

Prepare a tissue homogenate using an Isotonic Extraction Buffer, 25 mM HEPES, pH 7.4, with 1 mM EDTA and 250 mM sucrose is recommended.

1. Add 4 ml of Isotonic Extraction Buffer per 1 gram of washed tissue.
2. Homogenize the tissue with an Ultra-Turrax® T-25 homogenizer for 20 seconds and then centrifuge at 1,000 × *g*.
3. Discard the nuclear pellet and then centrifuge for 15 minutes at 16,000 × *g*. Use the supernatant for the assay.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C is recommended.

Procedure

The procedure should be performed in 96 well plates and read with a plate reader, or in a 160 µl cuvette with a 1 cm pathlength and read with a spectrophotometer. It is recommended to run all the samples in duplicates.

1. Set the plate reader at 450 nm and the temperature at 37 °C.
2. Program the plate reader to read every minute for 10 minutes.
3. Equilibrate the freshly prepared Working Solution at 37 °C in a constant temperature bath for at least 5 minutes.
4. Prepare the samples according to the Sample Preparation for Cell and Tissue extracts section.

Table 1.

Reaction/Standard Curve Schemes

Sample	Positive Control Solution	Unknown Sample	Assay Buffer	Working Solution
Blank	–	–	50 µl	150 µl
BVR positive control	4 µl	–	46 µl	150 µl
BVR positive control	8 µl	–	42 µl	150 µl
BVR positive control	12 µl	–	38 µl	150 µl
Test Sample	–	x µl (2–50 µl)	50 – x µl	150 µl

5. Place a suitable volume of unknown sample with a protein concentration in the range of 5–25 mg/ml in the wells (perform in duplicate):
   5–40 µl of the cell extracts or
   5–20 µl of the tissue extracts
   Note: In crude extracts the linearity of the reaction may be very poor if the sample size is too large due to the presence of endogenous inhibitors. Always assay several volumes of the unknown sample in order to find the linearity range of the reaction.
6. Bring the volume in the well to 50 µl with Assay Buffer. A blank will contain 50 µl of Assay Buffer alone.
7. Place in the preheated plate reader and allow equilibration to 37 °C for 2 minutes.
8. Start the reaction with 150 µl of the preheated Working Solution.
   Note: If there is bubble formation in the well after addition of the Working Solution, eliminate them before reading, by pricking with a 27-gauge needle.
9. Calculate the activity from the linear portion of the curve observed.

Calculation

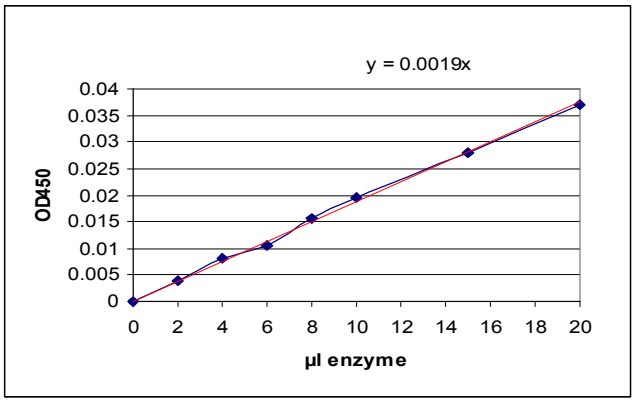
When working with biological extracts the activity should not be calculated over the whole time period, but only over the period of time, which gives a linear reaction rate. In concentrated solutions a short time period of 1 minute may suffice; whereas, in very dilute solutions an even longer time period is possible (up to 30 minutes).

$$\text{Units/ml} = \frac{(A_{sample} - A_{blank}) \times 1{,}000 \times 0.2}{53 \times V \times T \times L}$$

Where:
53 = extinction coefficient (ε^{mM}) of bilirubin at 450 nm
V = volume of enzyme sample (ml)
T = time of linear portion of the reaction (minutes)
L = pathlength of cell [cm] (1.0 in cuvette and 0.55 in 96 well plates)
0.2 = volume of reaction (ml)
1,000 = conversion factor to nanomole

Unit definition: 1 unit of biliverdin reductase will transform 1 nanomole of biliverdin to bilirubin in an NADPH dependent reaction at pH 8.5 at 37 °C.

Figure 1.
Typical Curve for the Positive Control Solution



References

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3. Maines, M.D., New insights into biliverdin reductase functions: linking heme metabolism to cell signaling. *Physiol*., **20**, 382-389 (2005).
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