



15-PGDH Inhibitor Screening Kit (Fluorometric)

05/16

(Catalog # K503-100; 100 assays; Store at -20°C)

I. Introduction:

15-hydroxyprostaglandin dehydrogenase (15-PGDH, EC: 1.1.1.141) catalyzes the oxidation of Prostaglandins (PG) to 15-keto metabolites which show greatly reduced biological activity when compared to Prostaglandins. 15-PGDH catalyzes the rate-limiting step of PG catabolism. More recently, the inhibition of 15-PGDH has been reported as a negative regulator of tissue repair and inhibition of this enzyme can potentiate tissue regeneration in mouse models. Thus, pharmacological inhibition of 15-PGDH could have clinical implications by accelerating potential tissue regeneration after damage. In this assay, 15-PG is oxidized by 15-PGDH generating 15-Keto metabolites and oxidizing NAD. NADH reduces a Fluorometric probe thus, generating an enhanced and stable fluorescence signal (Ex/Em=535/587 nm). In the presence of an inhibitor, the enzymatic activity is inhibited resulting No/Low fluorescence. BioVision's 15-PGDH Inhibitor Screening Kit offers a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of 15-PGDH inhibitors.



II. Application:

- Screening/studying/characterizing potential inhibitors of 15-hydroxyprostaglandin dehydrogenase

III. Kit Contents:

Components	K503-100	Cap Code	Part Number
15-PGDH Assay Buffer	25 ml	WM	K503-100-1
PicoProbe™ (in DMSO)	200 µl	Blue	K503-100-2
15-PGDH Developer	1 vial	Red	K503-100-3
15-PGDH Substrate (in DMSO)	100 µl	Purple	K503-100-4
15-PGDH Enzyme	25 µl	Green	K503-100-5
15-PGDH Inhibitor Control (in DMSO)	20 µl	Orange	K503-100-6

IV. User Supplied Reagents and Equipment:

- 96-well white opaque plate with flat bottom.
- Multi-well spectrophotometer (fluorescence plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **15-PGDH Assay Buffer:** Bring to room temperature before use. Store at -20°C. Use within two months.
- **PicoProbe™:** Before use, thaw at room temperature. Store at -20°C. Use within two months.
- **15-PGDH Developer:** Reconstitute with 220 µl of Assay Buffer. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.
- **15-PGDH Substrate and 15-PGDH Inhibitor Control:** Before use, thaw at room temperature. Store at -20°C. Use within two months.
- **15-PGDH Enzyme:** Ready to use. Keep on ice while in use. Store at -20°C. Use within two months.

VI. 15-PGDH Inhibitor Screening Protocol:

1. Screening Compounds, Inhibitor Control, Enzyme Control & Blank Control Preparations: Dissolve candidate inhibitors into an appropriate solvent (e.g. DMSO) at the highest concentration to be tested. Dilute to 2X desired test concentration with 15-PGDH Assay Buffer. Add 50 µl diluted test inhibitor or Assay Buffer into desired wells for Sample Compound [S], and Enzyme Control [EC] (no inhibitor) respectively. For 15-PGDH Inhibitor Control (IC), dilute 15-PGDH Inhibitor Control 200-fold by adding 2 µl Inhibitor Control to 398 µl 15-PGDH Assay Buffer. Add 50 µl of diluted Inhibitor Control into desired well(s). For reagent control, add 55 µl 15-PGDH Assay Buffer into one of wells.

Note:

- a. Prepare parallel well(s) as Solvent Control (SC) to test the effect of the solvent on 15-PGDH activity. In case SC is significantly different from EC, use its value to determine the effect of tested compound(s).
 - b. Do not store unused diluted 15-PGDH Inhibitor Control.
- 2. 15-PGDH Enzyme Preparation:** Make enough Diluted 15-PGDH Enzyme for the number of assays to be performed. Dilute 15-PGDH Enzyme 1:20 with Assay Buffer (e.g. add 10 µl of 15-PGDH Enzyme to 190 µl Assay Buffer; mix well). Add 5 µl of the diluted 15-PGDH Enzyme into Sample Compounds [S], Enzyme Control [EC], Solvent Control [SC] and Inhibitor Control(IC) wells. Incubate for 5 min. at 25°C.

Note:

The diluted 15-PGDH is stable for at least ~30 min. on ice. Discard the diluted 15-PGDH Enzyme after use. Always prepare a fresh diluted 15-PGDH Enzyme when needed.

3. Substrate Solution Preparation: Make enough reagents for the number of assays to be performed. For each well, prepare 45 µl of Substrate Solution Preparation containing:



	Reaction Mix
Assay Buffer	41 μ l
PicoProbe™	1 μ l
Developer	2 μ l
Substrate	1 μ l

Mix and add 45 μ l of Reaction Mix into each well (Sample Compound, Enzyme Control, reagent control, Solvent Control and Inhibitor Control). Mix well with gentle shaking.

Notes:

- a. Diluted substrate solution is stable for at least 20 min. on ice. Do not store the diluted substrate solution.
- b. Preset the plate reader to avoid delays in measurement after adding the substrate solution.
4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) kinetically at 25°C for 5-60 min. Choose two points (T_1 and T_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).
5. **Calculations:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU ($RFU_2 - RFU_1$) values by the time Δt ($t_2 - t_1$). Calculate % Relative Inhibition as follows. If the values of Solvent Control(s) are significantly different from the Enzyme Control, use SC values instead of EC values.

$$\text{Relative Activity (\%)} = \frac{\text{Slope of S}}{\text{Slope of EC}} \times 100$$

Where: **Slope of EC** is the enzyme Control Slope
Slope of S is the Sample Compound Slope

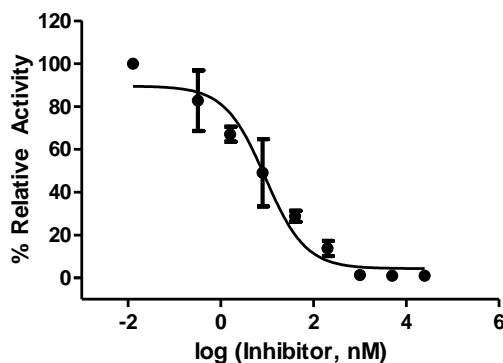


Figure: Inhibition of 15-PGDH Activity by 15-PGDH Inhibitor Control. IC_{50} was determined to be 9.1 +/- 0.1 nM. Assay was performed following the kit protocol.

VII. Related Products:

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| Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (K549) | COX-2 Inhibitor Screening Kit (K547) |
| Peroxidase Activity Assay Kit (K772) | Myeloperoxidase (MPO) Peroxidation Activity Assay Kit (K747) |
| Myeloperoxidase (MPO) Colorimetric Activity Assay Kit (K744) | Myeloperoxidase (MPO) Fluorometric Activity Assay Kit (K745) |
| Myeloperoxidase (MPO) Inhibitor Screening Kit (K746) | Celecoxib (1574) |

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