15-hydroxy Prostaglandin Dehydrogenase Substrate Screening Kit

Item No. 701980

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
701981	15-PGDH Assay Buffer (1X)	1 vial/12 ml	-20°C
701985	15-PGDH Enzyme (human, recombinant)	1 vial/50 µl	-80°C
701983	15-PGDH Positive Control (PGE_2)	1 vial/0.25 ml	-20°C
701984	15-PGDH Negative Control (15-keto PGE_2)	1 vial/0.25 ml	-20°C
700416	DTT (1 M) Assay Reagent	1 vial/1 ml	-20°C
701982	NAD Solution (100X)	1 vial/0.5 ml	-20°C
400115	Half-Area 96-Well Solid Plate (black, clear bottom)	1 plate	RT
400023	Foil Plate Cover	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence with excitation and emission wavelengths of 340 and 445 nm, respectively
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. Microcentrifuge tubes

INTRODUCTION

Background

15-hydroxy Prostaglandin dehydrogenase (15-PGDH) is an enzyme of the short-chain alcohol dehydrogenase family that catalyzes the oxidation of prostaglandins (PGs) to 15-keto metabolites with reduced biological activity.[1-4] It uses NAD^+ as a cofactor and, in addition to PGs, also converts various hydroxy fatty acids, such as HETEs, resolvins, and lipoxins to less active keto metabolites.[4-8] 15-PGDH acts as a tumor suppressor, and restoring *HPGD*, the gene encoding 15-PGDH, expression in human colon cancer cells *in vitro* reduces subsequent tumor formation in mouse xenograft models.[9-12] Knockout of *Hpgd* or inhibition of 15-PGDH in mice increases hepatic tissue regeneration and induces resistance to ulcerative colitis.[13] Inhibition of 15-PGDH also increases mitochondrial function and autophagic flux in muscle tissue, as well as increases muscle mass and strength in aged mice.[14] Identifying substrates of 15-PGDH is important for clarifying the roles of various bioactive compounds, which may or may not be structurally related to PGs and oxylipins, in cancer, inflammation, and other diseases.

About This Assay

Cayman's 15-PGDH Substrate Screening Assay Kit provides a robust and easy-to-use platform for identifying novel substrates of the metabolic enzyme 15-PGDH. Measurement of 15-PGDH activity is carried out by monitoring the rate of NAD^+ reduction to NADH (see Figure 1, below). The reduction of NAD^+ to NADH is accompanied by an increase in fluorescence at 445 nm following excitation at 340 nm. PGE_2 is included as a positive control while 15-keto PGE_2, an inactive metabolite of PGE_2 formed by 15-PGDH, is included as a negative (background) control. This assay is also able to detect "slow" substrates, which are utilized by 15-PGDH less efficiently than PGE_2 (see Figure 5, page 18).

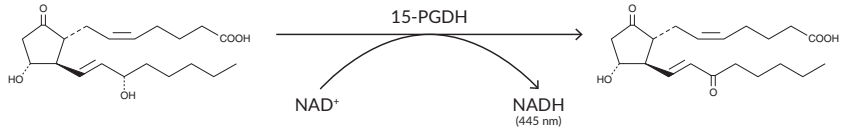


Figure 1. Assay Scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. Complete 15-PGDH Assay Buffer

Thaw the 15-PGDH Assay Buffer (1X) (Item No. 701981) and keep it at room temperature until use. Thaw the DTT (1 M) Assay Reagent (Item No. 700416) and NAD Solution (100X) (Item No. 701982) and keep on ice until use. Add 12 µl of DTT (1 M) Assay Reagent and 120 µl of NAD Solution (100X) to 15-PGDH Assay Buffer (1X). This is the Complete 15-PGDH Assay Buffer. Once thawed, any remaining incomplete 15-PGDH Assay Buffer (1X) may be stored at -20°C for 12 months and thawed and refrozen without effect. If all of the DTT (1 M) Assay Reagent and NAD Solution (100X) will not be used at one time, aliquot and store at -20°C. Avoid repeated freeze/thaw cycles.

2. 15-PGDH Positive Control (PGE_2) – (Item No. 701983)

This vial contains 0.25 ml of a 10 mM PGE_2 solution in ethanol to be used as a positive control. Mix 10 µl of 15-PGDH Positive Control (PGE_2) with 190 µl of Complete 15-PGDH Assay Buffer in a microcentrifuge tube. If all of the 15-PGDH Positive Control (PGE_2) will not be used at one time, aliquot the undiluted positive control and store at -20°C where it will be stable for 12 months.

3. 15-PGDH Negative Control (15-keto PGE_2) – (Item No. 701984)

This vial contains 0.25 ml of a 10 mM 15-keto PGE_2 solution in ethanol to be used as a negative control. Mix 10 µl of 15-PGDH Negative Control (15-keto PGE_2) with 190 µl of Complete 15-PGDH Assay Buffer in a microcentrifuge tube. If all of the 15-PGDH Negative Control (15-keto PGE_2) will not be used at one time, aliquot the undiluted negative control and store at -20°C where it will be stable for 12 months.

NOTE: Prepare the 15-PGDH Positive Control (PGE_2) and 15-PGDH Negative Control (15-keto PGE_2) before diluting the 15-PGDH (human, recombinant) enzyme.

4. 15-PGDH Enzyme (human, recombinant) – (Item No. 701985)

15-PGDH Enzyme (human, recombinant) should be thawed on ice and mixed prior to dilution. To dilute the enzyme, add the entire contents of the 15-PGDH (human, recombinant) vial to the remaining Complete 15-PGDH Assay Buffer. Mix thoroughly and keep at room temperature until used in the assay. It is recommended that the enzyme be diluted immediately prior to performing the assay. Discard any remaining diluted enzyme after the assay.

Sample Peparation

Dissolve the test substrates in either 100% ethanol or 100% dimethyl formamide (DMF) at a concentration of 500 µM. When added to the assay, the final concentration of substrate in the test wells will be 50 µM. If a different final concentration of substrates is desired, adjust the volumes used to dissolve the substrates accordingly.

NOTE: DMSO can also be used as a solvent but the signal-to-background ratio will be reduced (Figure 3, on page 15).

ASSAY PROTOCOL

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. There is no specific pattern for using the wells on the plate. It is recommended that three wells be designated for the 15-PGDH Positive Control (PGE_2) and three wells be designated for the 15-PGDH Negative Control (15-keto PGE_2). It is suggested that each test substrate be assayed in triplicate and that the contents of each well are recorded on the template sheet provided on page 21. A typical layout of samples to be measured in triplicate is provided below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	PC	7	7	7	15	15	15	23	23	23
B	NC	NC	NC	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

PC - Positive Control Wells
NC - Negative Control Wells
1-30 - Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 100 µl in all the wells.
- Use the Complete 15-PGDH Assay Buffer containing 15-PGDH Enzyme (human, recombinant) in the assay wells.
- All reagents must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in triplicate, but it is at the user's discretion to do so.
- 30 samples can be assayed in triplicate or 45 in duplicate.
- The assay is performed at room temperature.
- Monitor fluorescence at 445 nm with excitation at 340 nm.

Performing the Assay

NOTE: The addition of substrate initiates the reactions so it is critical to have all substrates (test substrates as well as the 15-PGDH Positive Control (PGE_2) and 15-PGDH Negative Control (15-keto PGE_2) ready to be added to the appropriate wells.

NOTE: The reactions occur quickly so it is critical to have a fluorescence plate reader available for use 15 minutes following substrate addition.

1. Add 90 µl of Complete 15-PGDH Assay Buffer containing 15-PGDH Enyzme (human, recombinant) to all wells being used in the assay (see Sample plate format, Figure 2, page 11).
2. **15-PGDH Negative Control (15-keto PGE_2) Wells**: add 10 µl of diluted 15-PGDH Negative Control (15-keto PGE_2) to the designated wells on the plate.
3. **15-PGDH Positive Control (PGE_2) Wells**: add 10 µl of diluted 15-PGDH Positive Control (PGE_2) to the designated wells on the plate.
4. **Test Substrate Wells**: add 10 µl of diluted test substrates to the designated wells on the plate.
5. Cover the plate with the Foil Plate Cover (Item No. 400023) and incubate at room temperature for 15 minutes.
6. Measure the fluorescence with excitation and emission wavelengths of 340 and 445 nm, respectively.

*If desired, the assay may be read kinetically rather than as an endpoint. Reading the assay kinetically may increase the signal-to-background ratio. The fluorescence should be measured at least once every minute at room temperature for 60 minutes. Determine the initial rate based on the linear portion of the kinetic curve. Calculations can be performed as shown below substituting initial rates for average fluorescence.

ANALYSIS

Calculations

1. Determine the mean fluorescence units for the 15-PGDH Positive Control (PGE_2) wells and set this as 100% activity.
2. Determine the mean fluorescence units for the 15-PGDH Negative Control (15-keto PGE_2) wells, as well as for the test substrate wells, and express the data as a percentage of the 15-PGDH Positive Control (PGE_2) activity.

Performance Characteristics

Effects of Solvents:

Compounds may be prepared in organic solvents such as DMF or short-chain alcohols, as long as the concentration of organic solvents in the assay is ≤5%. The proper vehicle control should be included in the assay.

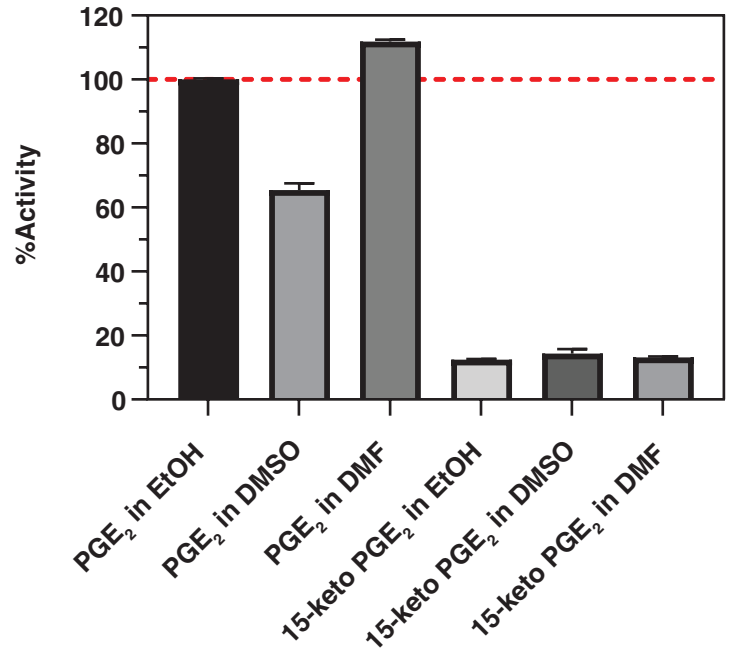


Figure 3. The effect of solvent on the readout of 15-PGDH activity. The data are shown as the mean ± standard deviation for sextuplicate reactions containing the indicated concentration of solvents.

Z´ Factor:

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[15]

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{| \mu_{c+} - \mu_{c-} |}$$

Where σ: Standard deviation
μ: Mean
c+: Positive control
c-: Negative control

The theoretical upper limit for the Z´ factor is 1.0. A robust assay has a Z´ factor >0.5. The Z´ factor for Cayman's 15-PGDH Substrate Screening Kit was determined to be 0.88.

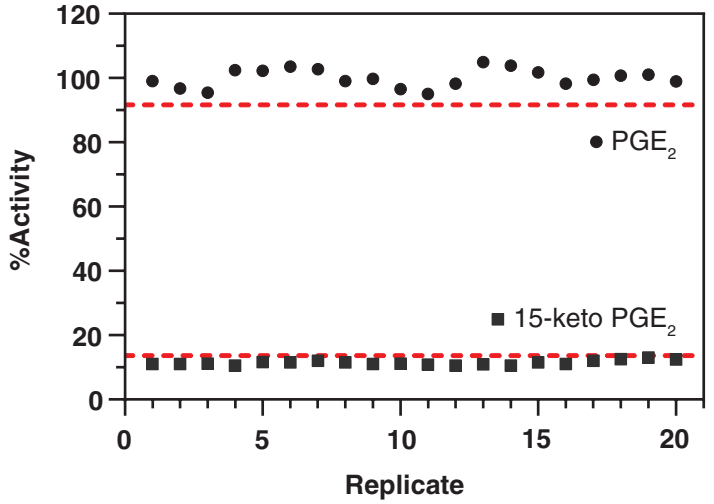


Figure 4. Typical Z′ data for the 15-PGDH Substrate Screening Kit. Data are shown from 20 replicates each for the Positive Control (PGE_2) and Negative Control (15-keto PGE_2) prepared as described in the kit booklet. The calculated Z′ factor for this experiment was 0.88. The red dotted lines correspond to three standard deviations from the mean for each control value.

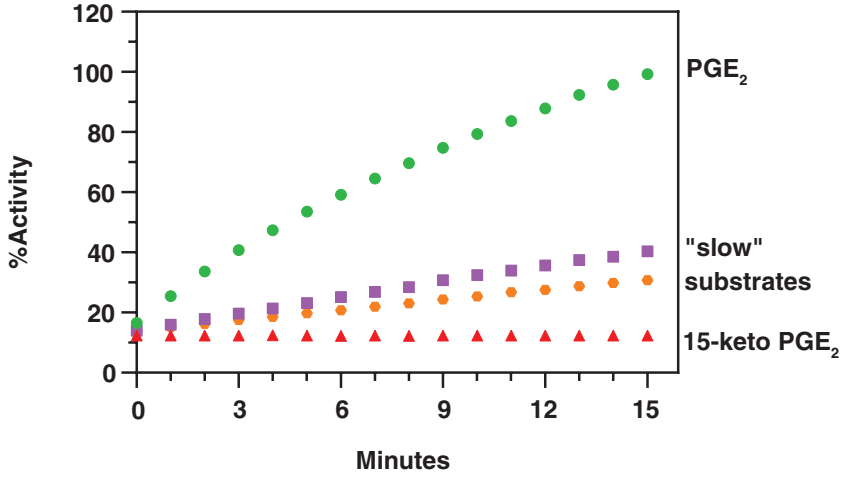


Figure 5. Utilization of "Slow" Substrates by 15-PGDH as Compared to Positive and Negative Control Substrates. Data are plotted as the mean of triplicate measurements ± the standard deviation. The Positive Control (PGE_2) represents 100% activity and the Negative Control (15-keto PGE_2) represents no activity.

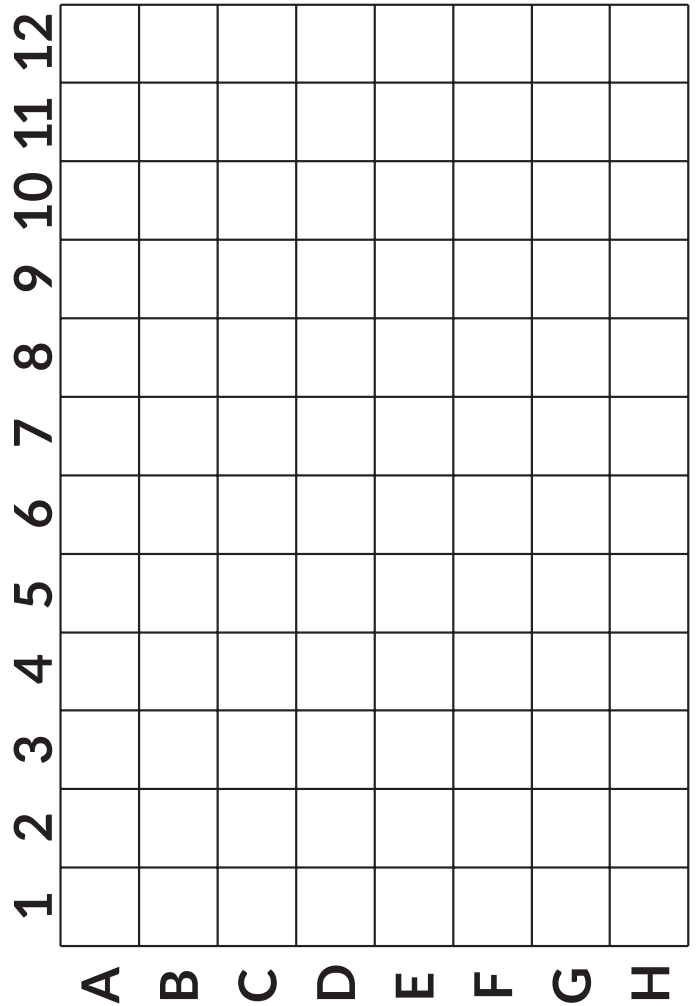
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No increase in fluorescence seen with the positive control	NAD and/or the enzyme were omitted from the assay buffer or no positive control was added to the wells.	Make sure to add both NAD and the enzyme to the assay buffer and make sure to add the positive control to the appropriate wells.

References

1. Jörnvall, H., Persson, B., Krook, M., *et al.* *Biochemistry* **34(18)**, 6003-6013 (1995).
2. Krook, M., Marekov, L., and Jörnvall, H. *Biochemistry* **29(3)**, 738-743 (1990).
3. Hansen, H.S. *Prostaglandins* **12(4)**, 647-679 (1976).
4. Ensor, C.M. and Tai, H.-H. *J. Lipid Mediat. Cell Signal* **12(2-3)**, 313-319 (1995).
5. Maddox, J.F., Colgan, S.P., Clish, C.B., *et al. FASEB Journal* **12(6)**, 487-494 (1998).
6. Sun, Y.P., Oh, S.F., Uddin, J., *et al. J. Biol. Chem.* **282(13)**, 9323-9334 (2007).
7. Di Marzo, V. *Rev. Physiol. Biochem. Pharmacol.* **160**, 1-24 (2006).
8. Hoffmann, F.M. and Maser, E. *Drug Metab. Rev.* **39(1)**, 87-144 (2007).
9. Wolf, I., O'Kelly, J., Rubinek, T., *et al. Cancer Res.* **66(15)**, 7818-7823 (2006).
10. Myung, S.J., Rerko, R.M., Yan, M., *et al. PNAS* **103(32)**, 12098-12102 (2006).
11. Yan, M., Rerko, R.M., Platzer, P., *et al. PNAS* **101(50)**, 17468-17473 (2004).
12. Pham, H., Chen, M., Li, A., *et al. Pancreas* **39(3)**, 332-339 (2010).
13. Zhang, Y., Desai, A., Yang, S.Y., *et al. Science* **348(6240)**, aaa2340 (2015).
14. Palla, A.R., Ravichandran, M., Wang, Y.X., *et al. Science* **371(6528)**, eabc8059 (2021).
15. Zhang, J.-H., Chung, T.D.Y., and Oldenburg, K.R. *J. Biomol. Screen.* **4(2)**, 67-73 (1999).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.