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## 15-hydroxy Prostaglandin Dehydrogenase Inhibitor Screening Assay Kit

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Item No. 702180

[www.caymanchem.com](http://www.caymanchem.com)  
Customer Service 800.364.9897  
Technical Support 888.526.5351  
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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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)	2 vials/12 ml	-20°C
702181	15-PGDH Enzyme (human, recombinant)	1 vial/25 µl	-80°C
702183	PGE <sub>2</sub> Substrate	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
702182	ML-148 Assay Reagent	1 vial/25 µl	-20°C
700001	DMSO Assay Reagent	1 vial/1 ml	RT
400091/400093	Half-Volume 96-Well Solid Plate (black)/ 384-Well Solid Plate (low volume; black)	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.

## 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. A source of ultrapure water, with a resistivity of 18.2 M $\Omega$ .cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Microcentrifuge tubes

## 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.<sup>1-4</sup> It uses NAD<sup>+</sup> 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.<sup>4-8</sup> 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.<sup>9-12</sup> Knockout of *Hpgd* or inhibition of 15-PGDH in mice increases hepatic tissue regeneration and induces resistance to ulcerative colitis.<sup>13</sup> 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.<sup>14</sup>

## About This Assay

Cayman's 15-PGDH Inhibitor Screening Assay Kit provides a robust and easy-to-use platform for identifying novel inhibitors of human 15-PGDH, the enzyme that catalyzes the oxidation of prostaglandins to 15-keto metabolites with reduced biological activity. Measurement of 15-PGDH activity is carried out by monitoring the reduction of NAD<sup>+</sup> to NADH. The reduction of NAD<sup>+</sup> to NADH is accompanied by an increase in fluorescence at 445 nm following excitation at 340 nm. The potent and reversible 15-PGDH inhibitor ML-148 Assay Reagent is included as a positive control and inhibits 15-PGDH with an IC<sub>50</sub> value of 11 nM.

## Sample Preparation

All inhibitors, be they small molecules, natural products, or proteins, should be prepared in Complete 15-PGDH Assay Buffer (1X) at a concentration 10X the desired final assay concentration (e.g., for 10 μM final assay concentration, a 100 μM stock should be made). This solution may contain up to 50% DMSO or short-chain alcohols (e.g., MeOH, EtOH). Dimethyl formamide (DMF) is not recommended as a solvent. The final concentration of organic solvents in the assay will then be ≤5% (see 'Effects of Solvents' on page 20).

## Reagent Preparation

### 1. Complete 15-PGDH Assay Buffer (1X)

Add 12 μl of DTT (1 M) Assay Reagent (Item No. 700416) to 12 ml of 15-PGDH Assay Buffer (1X) (Item No. 701981). This is the Complete 15-PGDH Assay Buffer (1X). 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. Complete 15-PGDH Assay Buffer (1X) is stable on ice for one day. If all of the DTT (1 M) Assay Reagent will not be used at one time, aliquot and store at -20°C, limiting freeze-thaw cycles.

### 2. PGE<sub>2</sub> Substrate

Mix 24 μL of PGE<sub>2</sub> Substrate (Item No. 702183) with 1.176 ml Complete 15-PGDH Assay Buffer (1X). This is enough substrate for 100 wells. The diluted substrate will be stable at room temperature for four hours. If all of the PGE<sub>2</sub> Substrate will not be used at one time, aliquot the undiluted substrate and store at -20°C where it will be stable for at least 12 months.

**3. 15-PGDH Enzyme (human, recombinant)**

15-PGDH Enzyme (human, recombinant) (Item No. 702181) should be thawed on ice and mixed prior to dilution. To dilute the enzyme, *gently* mix 13  $\mu$ l of 15-PGDH Enzyme (human, recombinant) with 1.247 ml Complete 15-PGDH Assay Buffer (1X) (*do not vortex!*). This is enough enzyme for 100 wells. It is recommended that the enzyme be diluted immediately prior to performing the assay. The diluted enzyme loses 20% of its activity when stored on ice for four hours. The undiluted enzyme can be aliquoted and stored at -80°C, limiting freeze-thaw cycles.

**4. ML-148 Diluent**

To make ML-148 Diluent, mix 125  $\mu$ l of DMSO Assay Reagent (Item No. 700001) with 375  $\mu$ l ultrapure water to make a 25% DMSO solution.

**5. ML-148 Assay Reagent**

This vial contains 25  $\mu$ l of 10 mM ML-148 Assay Reagent (Item No. 702182) in DMSO, which can be used as a positive control. Mix 5  $\mu$ l of ML-148 Assay Reagent with 495  $\mu$ l of ML-148 Diluent to make a 100  $\mu$ M working solution. If all of the ML-148 Assay Reagent will not be used at one time, aliquot the undiluted inhibitor and store at -20°C.

**6. NAD Solution (100X)**

This vial contains NAD solution (100X). Mix 120  $\mu$ l of NAD solution (100X) with 1.080 ml of Complete 15-PGDH Assay Buffer (1X) to prepare a 10X working solution. This is enough 10X solution for 100 wells. If all of the NAD solution (100X) will not be used at one time, aliquot the undiluted NAD solution (100X) and store at -20°C, limiting freeze-thaw cycles.

**Sample Preparation**

All inhibitors, be they small molecules, natural products, or proteins, should be prepared in Complete 15-PGDH Assay Buffer (1X) at a concentration 5X the desired final assay concentration (e.g., for 10  $\mu$ M final assay concentration, a 50  $\mu$ M stock should be made). This solution may contain up to 25% DMSO or short-chain alcohols (e.g., MeOH, EtOH). DMF is not recommended as a solvent. The final concentration of organic solvents in the assay will then be  $\leq$ 5% (see 'Effects of Solvents' on page 20).

**Reagent Preparation****1. Complete 15-PGDH Assay Buffer (1X)**

Add 12  $\mu$ l of DTT (1 M) Assay Reagent (Item No. 700416) to 12 ml of 15-PGDH Assay Buffer (1X) (Item No. 701981). This is the Complete 15-PGDH Assay Buffer (1X). 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. Complete 15-PGDH Assay Buffer (1X) is stable on ice for one day. If all of the DTT (1 M) Assay Reagent will not be used at one time, aliquot and store at -20°C, limiting freeze-thaw cycles.

**2. PGE<sub>2</sub> Substrate**

Mix 24  $\mu$ l PGE<sub>2</sub> Substrate (Item No. 702183) with 2.376 ml Complete 15-PGDH Assay Buffer (1X). This is enough substrate for 400 wells. The diluted substrate will be stable at room temperature for four hours. If all of the PGE<sub>2</sub> Substrate will not be used at one time, aliquot the undiluted substrate and store at -20°C where it will be stable for at least 12 months.

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. However, it is necessary to have three wells designated as 100% initial activity and three wells designated as background. It is suggested that each inhibitor, including the positive control ML-148 Assay Reagent, be assayed in triplicate. It is suggested that the contents of each well be recorded on the template sheet provided on page 25. A typical layout of samples to be measured in triplicate is shown in Figure 1, below.

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

BW - Background Wells  
 A - 100% Initial Activity Wells  
 PC - Positive Control Wells  
 1-29 - Inhibitor Wells

Figure 1. Sample plate format

3. 15-PGDH Enzyme (human, recombinant)

15-PGDH Enzyme (human, recombinant) (Item No. 702181) should be thawed on ice and mixed prior to dilution. To dilute the enzyme, *gently* mix 13 µl of 15-PGDH Enzyme (human, recombinant) with 2.507 ml Complete 15-PGDH Assay Buffer (1X) (*do not vortex!*). This is enough enzyme for 400 wells. It is recommended that the enzyme be diluted immediately prior to performing the assay. The diluted enzyme loses 20% of its activity when stored on ice for four hours. The undiluted enzyme can be aliquoted and stored at -80°C, limiting freeze-thaw cycles.

4. ML-148 Diluent

To make ML-148 Diluent, mix 125 µl of DMSO Assay Reagent (Item No. 700001) with 875 µl ultrapure water to make a 12.5% DMSO solution.

5. ML-148 Assay Reagent

This vial contains 25 µl of 10 mM ML-148 Assay Reagent (Item No. 702182) in DMSO, which can be used as a positive control. Mix 5 µl of ML-148 Assay Reagent with 995 µl of ML-148 Diluent to make a 50 µM working solution. If all of the ML-148 Assay Reagent will not be used at one time, aliquot the undiluted inhibitor and store at -20°C.

6. NAD Solution (100X)

This vial contains NAD solution (100X). Mix 100 µl of NAD solution (100X) with 1.900 ml of Complete 15-PGDH Assay Buffer (1X) to prepare a 5X working solution. This is enough 5X solution for 400 wells. If all of the NAD solution (100X) will not be used at one time, aliquot and store at -20°C, limiting freeze-thaw cycles.

### 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  $\mu\text{l}$  in all the wells.
- Use the complete 15-PGDH Assay Buffer (1X) in the assay.
- All reagents should be prepared as described above. The 15-PGDH enzyme should be kept on ice and all other reagents should be kept at 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.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 340 nm and an emission wavelength of 445 nm.

### Performing the Assay

1. **Background Wells:** add 70  $\mu\text{l}$  Complete 15-PGDH Assay Buffer (1X) and 10  $\mu\text{l}$  of solvent (the same solvent concentration used to dissolve the unknown inhibitor and the positive control, ML-148 Assay Reagent, to three wells. If inhibitors in different solvents are to be assayed at the same time, separate sets of background wells should be run for each solvent.
2. **100% Initial Activity Wells:** add 60  $\mu\text{l}$  Complete 15-PGDH Assay Buffer (1X), 10  $\mu\text{l}$  15-PGDH Enzyme, and 10  $\mu\text{l}$  of solvent to three wells. If inhibitors in different solvents are to be assayed at the same time, separate sets of 100% initial activity wells should be run for each solvent.
3. **Inhibitor/Positive Control Wells:** add 60  $\mu\text{l}$  Complete 15-PGDH Assay Buffer (1X), 10  $\mu\text{l}$  of 15-PGDH Enzyme, and 10  $\mu\text{l}$  of unknown inhibitor or the 100  $\mu\text{M}$  positive control, ML-148 Assay Reagent working solution, to three wells. *NOTE: To determine an  $IC_{50}$  value for an inhibitor, multiple concentrations of the inhibitor should be tested in the assay.*
4. Incubate for 10 minutes at room temperature.
5. Add 10  $\mu\text{l}$  of the diluted PGE<sub>2</sub> Substrate to all wells being used
6. Initiate the reactions by adding 10  $\mu\text{l}$  of NAD working solution to all the wells being used. Mixing the contents is not necessary.
7. Cover the plate with the Foil Plate Cover (Item No. 400023) and incubate for 30 minutes at room temperature.
8. Remove the plate cover and read the plate with an excitation wavelength of 340 nm and an emission wavelength of 445 nm. It may be necessary to adjust the gain setting to allow for the measurement of all samples.\*

\*If desired, the assay may be read kinetically rather than as an endpoint. Reading the assay kinetically may increase signal-to-background. The fluorescence should be measured at least once per minute at room temperature for 30 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.



## ASSAY PROTOCOL FOR 384-WELL ASSAY

### Plate Set Up

The 384-well plate(s) included with this kit is supplied ready to use. There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity and three wells designated as background. It is suggested that each inhibitor, including the positive control ML-148 Assay Reagent, be assayed in triplicate.

#### Pipetting Hints

- It is recommended that an 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 25  $\mu$ l in all the wells.
- Use the complete 15-PGDH Assay Buffer (1X) in the assay.
- All reagents should be prepared as described above. The 15-PGDH enzyme should be kept on ice and all other reagents should be kept at 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.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 340 nm and an emission wavelength of 445 nm.

### Performing the Assay

1. **Background Wells:** add 10  $\mu$ l Complete 15-PGDH Assay Buffer (1X) and 5  $\mu$ l of solvent (the same solvent concentration used to dissolve the unknown inhibitor and the positive control, ML-148 Assay Reagent, to three wells. If inhibitors in different solvents are to be assayed at the same time, separate sets of background wells should be run for each solvent
2. **100% Initial Activity Wells:** add 5  $\mu$ l Complete 15-PGDH Assay Buffer (1X), 5  $\mu$ l of 15-PGDH Enzyme, and 5  $\mu$ l of solvent to three wells. If inhibitors in different solvents are to be assayed at the same time, separate sets of 100% initial activity wells should be run for each solvent.
3. **Inhibitor/Positive Control Wells:** add 5  $\mu$ l Complete 15-PGDH Assay Buffer (1X), 5  $\mu$ l of 15-PGDH Enzyme, and 5  $\mu$ l of unknown inhibitor or the 50  $\mu$ M positive control, ML-148 Assay Reagent working solution, to three wells. *NOTE: To determine an  $IC_{50}$  value for an inhibitor, multiple concentrations of the inhibitor should be tested in the assay.*
4. Incubate for 10 minutes at room temperature.
5. Add 5  $\mu$ l of the diluted PGE<sub>2</sub> Substrate to all wells being used.
6. Initiate the reactions by adding 5  $\mu$ l of NAD working solution to all the wells being used. Mixing the contents is not necessary.
7. Cover the plate with the Foil Plate Cover (Item No. 400023) and incubate for 30 minutes at room temperature.
8. Remove the plate cover and read the plate with an excitation wavelength of 340 nm and an emission wavelength of 445 nm. It may be necessary to adjust the gain setting to allow for the measurement of all samples.\*

\*If desired, the assay may be read kinetically rather than as an endpoint. Reading the assay kinetically may increase signal-to-background. The fluorescence should be measured at least once per minute at room temperature for 30 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.

## Calculations

1. Determine the average fluorescence (AF) of each sample.
2. Subtract the AF of the background wells from the AF of the 100% initial activity and inhibitor wells. These are the corrected values.
3. Determine the percent inhibition or percent activity for each inhibitor using one of the following equations:

$$\% \text{ inhibition} = \left[ \frac{(\text{corrected 100\% initial activity} - \text{corrected inhibitor activity})}{\text{corrected 100\% initial activity}} \right] \times 100$$

$$\% \text{ activity} = \left[ \frac{(\text{corrected inhibitor activity})}{\text{corrected 100\% initial activity}} \right] \times 100$$

4. Graph the percent inhibition or percent activity as a function of inhibitor concentration to determine the  $IC_{50}$  value (the concentration at which there is 50% inhibition) of the inhibitor. Inhibition of recombinant human 15-PGDH by ML-148 Assay Reagent is shown in Figure 2 (see page 18).

## Performance Characteristics

### Z' Factor:

Z' factor is a term used to describe the robustness of an assay, which is calculated using the equation below.<sup>15</sup>

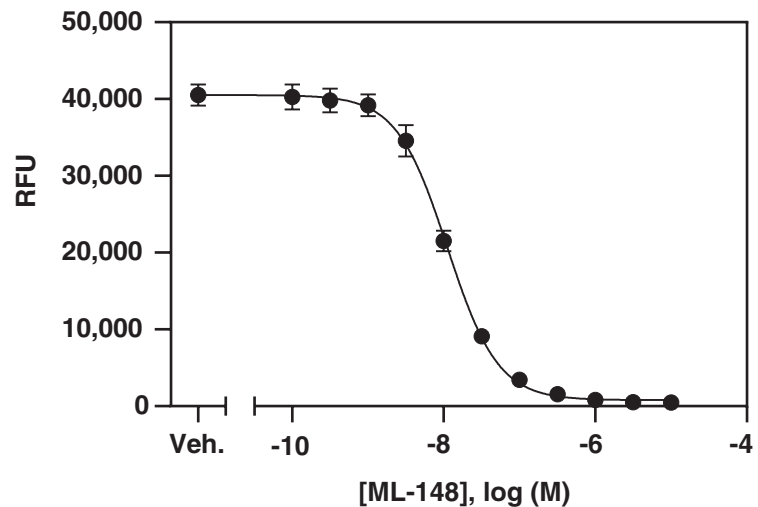
$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

Where  $\sigma$ : Standard deviation  
 $\mu$ : Mean  
 c+: Positive control  
 c-: Negative control

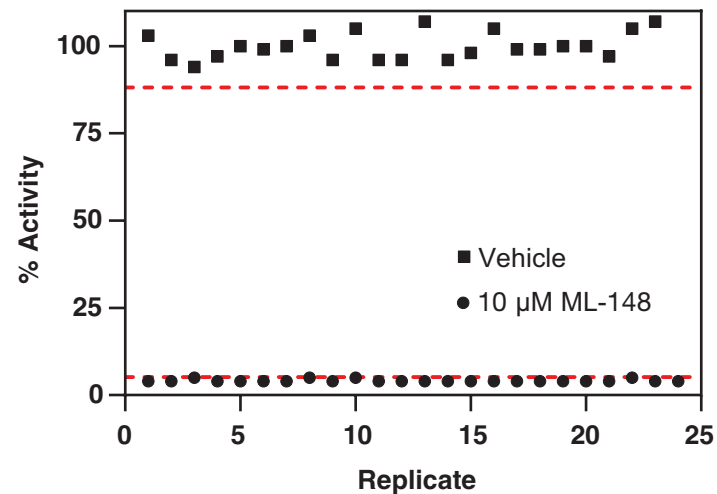
The theoretical upper limit for the Z' factor is 1.0. A robust assay has a Z' factor >0.5. The Z' factor for Cayman's 15-PGDH Inhibitor Screening Assay Kit was determined to be 0.82 for a 96-well plate and 0.87 for 384-well plate.

### Sample Data:

The data presented here are examples of data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.



**Figure 2. Inhibition of recombinant human 15-PGDH by ML-148 Assay Reagent.** Data are plotted as the mean of triplicate measurements  $\pm$  the standard deviation. The vehicle control (Veh.) represents 100% initial activity. The  $IC_{50}$  value of ML-148 Assay Reagent is 11 nM.



**Figure 3. Typical  $Z'$  data for the 15-PGDH Inhibitor Screening Assay Kit.** Data are shown from 24 replicates each for vehicle control (Veh.) and 10  $\mu$ M ML-148 Assay Reagent prepared as described in the kit booklet and run in the 384-well format. The calculated  $Z'$  factor for this experiment was 0.87. The red lines correspond to three standard deviations from the mean for each control value.

### Effects of Solvents:

Compounds may be prepared in organic solvents such as DMSO or short-chain alcohols (e.g., MeOH, EtOH), as long as the final concentration of organic solvents in the assay is  $\leq 5\%$ . DMF is not recommended as a solvent. A titration of organic solvents showed that signal can vary with increasing solvent concentration so the proper vehicle control should be included in the assay.

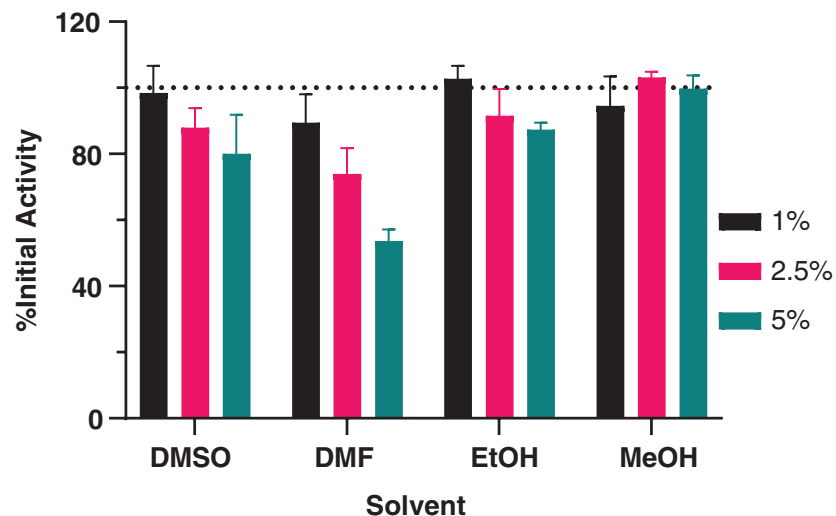


Figure 4. The effect of solvent on the readout of 15-PGDH activity. The data are shown as the mean  $\pm$  standard deviation for triplicate reactions containing the indicated concentration of solvents. The dotted line at 100% is the buffer control.

### Precision:

Intra-assay precision was determined by analyzing 7, 24, and 24 measurements of the background, vehicle, and 100  $\mu\text{M}$  ML-148 Assay Reagent on the same day. The intra-assay coefficients of variation were 3, 5, and 3%, respectively. The intra-assay coefficient of variation for the  $\text{IC}_{50}$  value of seven inhibition curves performed on the same day was 17%.

Inter-assay precision was determined by analyzing inhibition with ML-148 Assay Reagent in separate assays on four different days. The inter-assay coefficient of variance for the  $\text{IC}_{50}$  value was 19%.

### Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence detected above background in the inhibitor wells	A. Enzyme or substrate was not added to the wells B. Inhibitor concentration is too high and inhibited all of the enzyme activity	A. Make sure to add all the components to the well(s) and re-assay B. Reduce the inhibitor concentration and re-assay
The fluorometer exhibited 'MAX' values for the wells	The <i>gain</i> setting is too high	Reduce the <i>gain</i> and re-read
No inhibition seen with compound	A. The compound concentration is not high enough B. The compound is not an inhibitor of the enzyme	Increase the compound concentration and re-assay

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### 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.

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