ENPP1 Fluorescent Inhibitor Screening Assay Kit

Item No. 702090

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 kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
702091	ENPP1 Assay Buffer (10X)	1 vial/5 ml	-20°C
702092	ENPP1 Enzyme (human, recombinant)	1 vial/40 µl	-80°C
702093	ENPP1 Substrate (TG-mAMP)	1 vial/250 µl	-20°C
702094	ENPP1 Inhibitor C Assay Reagent	1 vial/50 µl	-20°C
400091 OR 400093	Half-Volume 96-Well Solid Plate (black) OR 384-Well Solid Plate (low volume; black)	1 plate	RT
400012	96-Well Cover Sheet	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 485 and 520 nm, respectively
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·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

INTRODUCTION

Background

Ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1) is a type II transmembrane glycoprotein with nucleotide pyrophosphatase and phosphodiesterase enzyme activities.[1] It is expressed in skeletal, adipose, hepatic, renal, cardiac, and placental tissues, as well as pancreatic islets, salivary glands, chondrocytes, fibroblasts, and lymphocytes.[2] ENPP1 is a critical regulator of purinergic signaling that catalyzes the hydrolysis of ATP or GTP to AMP or GMP, respectively, as well as the hydrolysis of cGAMP, and has roles in platelet aggregation, muscle contraction, hypoxia, ischemia, cGAS-STING signaling, and cell proliferation, migration, apoptosis, and differentiation.[1,3] This nucleotide hydrolysis also generates inorganic pyrophosphates, which inhibit bone and cartilage mineralization, implicating ENPP1 as a regulator of bone and cartilage development. Loss-of-function mutations in *ENPP1* are associated with various calcification-related disorders, including autosomal recessive hypophosphatemic rickets type 2 (ARHR2) generalized arterial calcification of infancy (GACI), and pseudoxanthoma elasticum (PXE).[1,4] *ENPP1* SNPs are associated with insulin resistance and the development of diabetic nephropathy.[2,5] ENPP1 hydrolysis of cGAMP in the tumor microenvironment decreases STING activation, facilitates immunosuppression, and is associated with enhanced bone metastasis in mouse xenograft models of breast cancer.[1,3] Inhibition of ENPP1 promotes STING activation and increases survival in various mouse xenograft models, indicating the potential therapeutic utility of ENPP1 inhibition in cancer.[1]

About This Assay

Cayman's ENPP1 Inhibitor Screening Assay Kit provides a robust and easy-to-use platform for identifying novel inhibitors of human ENPP1, an ectonucleotide pyrophosphatase and phosphodiesterase implicated in multiple biological pathways, including the cGAS/STING pathway, and bone mineralization. The assay uses an ENPP1-specific fluorogenic substrate, Tokyo Green™-mAMP (TG-mAMP). ENPP1 cleaves this substrate generating free Tokyo Green™, which can be easily quantified using a fluorescence plate reader at excitation and emission wavelengths of 485 and 520 nm, respectively. The potent and reversible ENPP1 inhibitor ENPP1 Inhibitor C Assay Reagent is included as a positive control.

PRE-ASSAY PREPARATION FOR 96-WELL ASSAY

Sample Preparation

All inhibitors, be they small molecules, natural products, or proteins, should be prepared in diluted ENPP1 Assay Buffer at a concentration 10X the desired final assay concentration (*e.g.*, for 30 µM final assay concentration, a 300 µM stock should be made). This solution may contain up to 20% 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 ≤2% (see 'Effects of Solvents' on page 24).

Reagent Preparation

1. **ENPP1 Assay Buffer (1X)**

   Mix 2 ml of ENPP1 Assay Buffer (10X) (Item No. 702091) with 18 ml of ultrapure water to make 20 ml of ENPP1 Assay Buffer (1X). The ENPP1 Assay Buffer (1X) should be discarded if not used within the same day. Once thawed, the ENPP1 Assay Buffer (10X) may be stored at 4°C.

2. **ENPP1 Substrate (TG-mAMP)**

   Mix 200 µl ENPP1 Substrate (TG-mAMP) (Item No. 702093) with 1.8 ml ENPP1 Assay Buffer (1X). The diluted substrate will be stable at room temperature (RT) for four hours. If all of the ENPP1 Substrate (TG-mAMP) will not be used at one time, aliquot the undiluted substrate and store at -20°C.

3. **ENPP1 Enzyme (human, recombinant)**

   ENPP1 Enzyme (human, recombinant) (Item No. 702092) should be thawed on ice and mixed prior to dilution. To dilute the enzyme, mix 25 µl of ENPP1 Enzyme (human, recombinant) with 1.975 ml ENPP1 Assay Buffer (1X). It is recommended that the enzyme be diluted immediately prior to performing the assay. The diluted enzyme loses 30% of its activity when stored on ice for one hour. The undiluted enzyme can be stored at -80°C, limiting freeze-thaw cycles.

4. **ENPP1 Inhibitor C Assay Reagent**

   This vial contains 50 µl of 6 mM ENPP1 Inhibitor C Assay Reagent (Item No. 702094) in DMSO, which can be used as a positive control. Mix 5 µl of ENPP1 Inhibitor C Assay Reagent with 95 µl ENPP1 Assay Buffer (1X) to make a 300 µM working solution. If all of the ENPP1 Inhibitor C Assay Reagent will not be used at one time, aliquot the undiluted inhibitor and store at -20°C, limiting freeze-thaw cycles.

PRE-ASSAY PREPARATION FOR 384-WELL ASSAY

Sample Preparation

All inhibitors, be they small molecules, natural products, or proteins, should be prepared in diluted ENPP1 Assay Buffer at a concentration 5X the desired final assay concentration (*e.g.*, for 30 µM final assay concentration, a 150 µM stock should be made). This solution may contain up to 10% 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 ≤2% (see 'Effects of Solvents' on page 24).

Reagent Preparation

1. **ENPP1 Assay Buffer (1X)**

   Mix 2 ml of ENPP1 Assay Buffer (10X) (Item No. 702091) with 18 ml of ultrapure water to make 20 ml of ENPP1 Assay Buffer (1X). The ENPP1 Assay Buffer (1X) should be discarded if not used within the same day. Once thawed, the ENPP1 Assay Buffer (10X) may be stored at 4°C.

2. **ENPP1 Substrate (TG-mAMP)**

   Mix 200 µl ENPP1 Substrate (TG-mAMP) (Item No. 702093) with 3.8 ml ENPP1 Assay Buffer (1X). The diluted substrate will be stable at RT for four hours. If all of the ENPP1 Substrate (TG-mAMP) will not be used at one time, aliquot the undiluted substrate and store at -20°C.

3. **ENPP1 Enzyme (human, recombinant)**

   ENPP1 Enzyme (human, recombinant) (Item No. 702092) should be thawed on ice and mixed prior to dilution. To dilute the enzyme, mix 25 µl of ENPP1 Enzyme (human, recombinant) with 3.975 ml ENPP1 Assay Buffer (1X). It is recommended that the enzyme be diluted immediately prior to performing the assay. The diluted enzyme loses 30% of its activity when stored on ice for one hour. The undiluted enzyme can be stored at -80°C, limiting freeze-thaw cycles.

4. **ENPP1 Inhibitor C Assay Reagent**

   This vial contains 50 µl of 6 mM ENPP1 Inhibitor C Assay Reagent (Item No. 702094) in DMSO, which can be used as a positive control. Mix 5 µl of ENPP1 Inhibitor C Assay Reagent with 195 µl ENPP1 Assay Buffer (1X) to make a 150 µM working solution. If all of the ENPP1 Inhibitor C Assay Reagent will not be used at one time, aliquot the undiluted inhibitor and store at -20°C, limiting freeze-thaw cycles.

ASSAY PROTOCOL FOR 96-WELL ASSAY

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 ENPP1 Inhibitor C Assay Reagent, be assayed in triplicate. It is suggested that the contents of each well be recorded on the template sheet provided on page 29. 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 96-well 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 diluted assay buffer in the assay.
- All reagents should be prepared as described above. The ENPP1 enzyme should be kept on ice and all other reagents should be kept at RT 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 RT.
- Monitor the fluorescence with an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

Performing the Assay

1. **Background Wells:** add 70 µl ENPP1 Assay Buffer (1X) and 10 µl of solvent (the same solvent concentration used to dissolve the unknown inhibitor and the positive control, ENPP1 Inhibitor C Assay Reagent) to three wells. If 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 50 µl of ENPP1 Assay Buffer (1X), 20 µl of ENPP1 Enzyme, and 10 µl 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 50 µl ENPP1 Assay Buffer (1X), 20 µl of ENPP1 Enzyme, and 10 µl of unknown inhibitor or the 300 µM positive control, ENPP1 Inhibitor C 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 5 minutes at RT.
5. Initiate the reactions by adding 20 µl of ENPP1 Substrate (TG-mAMP) to all the wells being used. Mixing the contents is not necessary.
6. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for twenty minutes at RT protected from light.
7. Remove the plate cover and read the plate with an excitation wavelength of 485 nm and an emission wavelength of 520 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 RT for 30 minutes. Determine the initial rate based on the linear portion of the kinetic curve. Calculations can be performed as shown on page 20 substituting initial rates for average fluorescence.

	100% Initial Activity	Positive Control (Inhibitor C)	Unknown Inhibitor	Background
Assay Buffer (1X)	50 µl	50 µl	50 µl	70 µl
Solvent	10 µl	--	--	10 µl
Positive Control (Inhibitor C)	--	10 µl	--	--
Unknown Inhibitor	--	--	10 µl	--
ENPP1	20 µl			--
Incubate	Incubate 5 minutes at RT			
Substrate	20 µl			
Incubate	Incubate for 20 minutes at RT protected from light			
Read	Read the plate at ex./em. = 485/520 nm			

Table 1. ENPP1 96-well plate format assay summary

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 ENPP1 Inhibitor C Assay Reagent, be assayed in triplicate. A typical layout of samples to be measured in triplicate is shown in Figure 2, below.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	BW	BW	BW	14	14	14	30	30	30	46	46	46	62	62	62	78	78	78	94	94	94	110	110	110
B	A	A	A	15	15	15	31	31	31	47	47	47	63	63	63	79	79	79	95	95	95	111	111	111
C	PC	PC	PC	16	16	16	32	32	32	48	48	48	64	64	64	80	80	80	96	96	96	112	112	112
D	1	1	1	17	17	17	33	33	33	49	49	49	65	65	65	81	81	81	97	97	97	113	113	113
E	2	2	2	18	18	18	34	34	34	50	50	50	66	66	66	82	82	82	98	98	98	114	114	114
F	3	3	3	19	19	19	35	35	35	51	51	51	67	67	67	83	83	83	99	99	99	115	115	115
G	4	4	4	20	20	20	36	36	36	52	52	52	68	68	68	84	84	84	100	100	100	116	116	116
H	5	5	5	21	21	21	37	37	37	53	53	53	69	69	69	85	85	85	101	101	101	117	117	117
I	6	6	6	22	22	22	38	38	38	54	54	54	70	70	70	86	86	86	102	102	102	118	118	118
J	7	7	7	23	23	23	39	39	39	55	55	55	71	71	71	87	87	87	103	103	103	119	119	119
K	8	8	8	24	24	24	40	40	40	56	56	56	72	72	72	88	88	88	104	104	104	120	120	120
L	9	9	9	25	25	25	41	41	41	57	57	57	73	73	73	89	89	89	105	105	105	121	121	121
M	10	10	10	26	26	26	42	42	42	58	58	58	74	74	74	90	90	90	106	106	106	122	122	122
N	11	11	11	27	27	27	43	43	43	59	59	59	75	75	75	91	91	91	107	107	107	123	123	123
O	12	12	12	28	28	28	44	44	44	60	60	60	76	76	76	92	92	92	108	108	108	124	124	124
P	13	13	13	29	29	29	45	45	45	61	61	61	77	77	77	93	93	93	109	109	109	125	125	125

BW - Background Wells
A - 100% Initial Activity Wells
PC - Positive Control Wells
1-125 - Inhibitor Wells

Figure 2. Sample 384-well 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 25 µl in all the wells.
- Use the diluted assay buffer in the assay.
- All reagents should be prepared as described above. The ENPP1 enzyme should be kept on ice and all other reagents should be kept at RT 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 RT.
- Monitor the fluorescence with an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

Performing the Assay

1. **Background Wells**: add 10 µl ENPP1 Assay Buffer (1X) and 5 µl of solvent (the same solvent concentration used to dissolve the unknown inhibitor and the positive control, ENPP1 Inhibitor C Assay Reagent) to three wells. If 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 10 µl of ENPP1 Enzyme and 5 µl 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 10 µl of ENPP1 Enzyme and 5 µl of unknown inhibitor or the 150 µM positive control, ENPP1 Inhibitor C 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 5 minutes at RT.
5. Initiate the reactions by adding 10 µl of ENPP1 Substrate (TG-mAMP) to all the wells being used. Mixing the contents is not necessary.
6. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for twenty minutes at RT protected from light.
7. Remove the plate cover and read the plate with an excitation wavelength of 485 nm and an emission wavelength of 520 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 RT for 30 minutes. Determine the initial rate based on the linear portion of the kinetic curve. Calculations can be performed as shown on page 20 substituting initial rates for average fluorescence.

	100% Initial Activity	Positive Control (Inhibitor C)	Unknown Inhibitor	Background
Assay Buffer (1X)	--	--	--	10 µl
Solvent	5 µl	--	--	5 µl
Positive Control (Inhibitor C)	--	5 µl	--	--
Unknown Inhibitor	--	--	5 µl	--
ENPP1	10 µl			--
Incubate	Incubate 5 minutes at RT			
Substrate	10 µl			
Incubate	Incubate for 20 minutes at RT protected from light			
Read	Read the plate at ex./em. = 485/520 nm			

Table 2. ENPP1 384-well plate format assay summary

ANALYSIS

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 ENPP1 by ENPP1 Inhibitor C Assay Reagent is shown in Figure 3 (see page 22).

Performance Characteristics

Z´ Factor:

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[6]

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

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 ENPP1 Fluorescent Inhibitor Screening Assay Kit was determined to be 0.82 for a 96-well plate and 0.79 for a 384-well plate.

Sample Data:

The data shown here is an example of the 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.

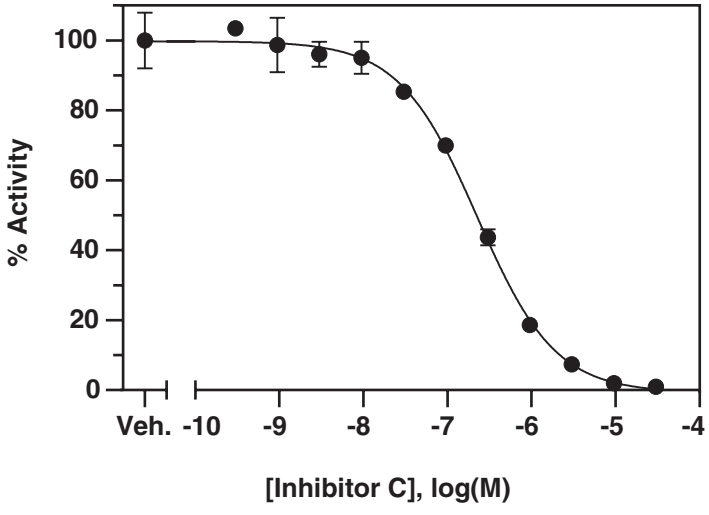


Figure 3. Inhibition of recombinant human ENPP1 by ENPP1 Inhibitor C Assay Reagent. Data are plotted as the mean of triplicate measurements ± the standard deviation. The vehicle control (Veh.) represents 100% initial activity.

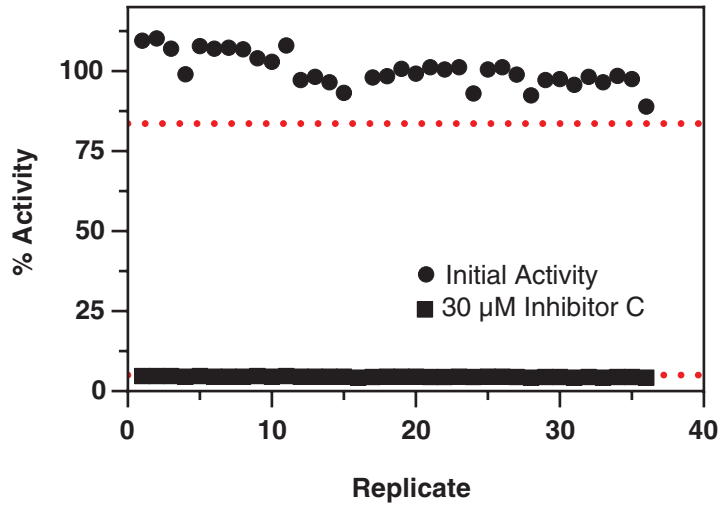


Figure 4. Typical Z' data for the ENPP1 Fluorescent Inhibitor Screening Assay Kit. Data are shown from 36 replicates each for initial activity and 30 µM ENPP1 Inhibitor C Assay Reagent prepared as described in the kit booklet. The calculated Z' factor for this experiment was 0.82 for a 96-well plate and 0.79 for a 384-well plate. 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 ≤2%. A titration of organic solvents showed that the signal changes with increasing solvent concentration, so the proper vehicle control should be included in the assay.

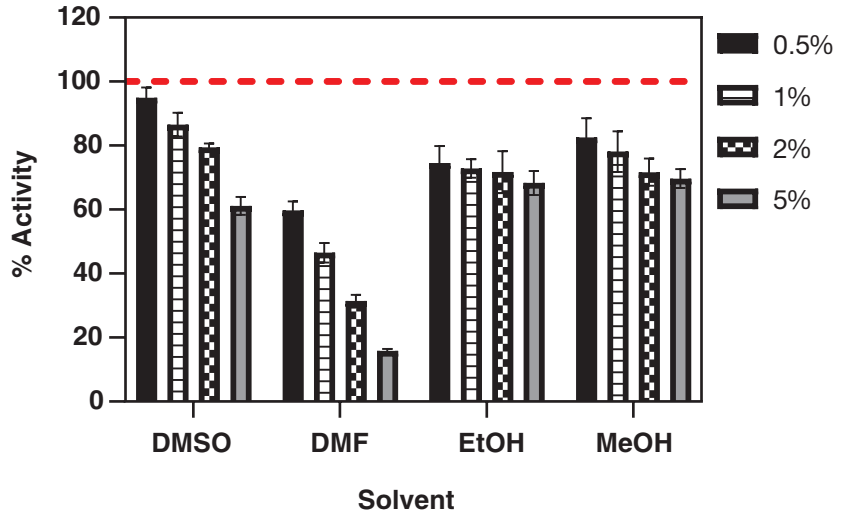


Figure 5. The effect of solvent on the readout of ENPP1 activity. The data are shown as the mean ± standard deviation for triplicate reactions containing the indicated concentration of solvents. The red dotted line at 100% is the buffer control.

Precision:

Intra-assay precision was determined by analyzing 3, 7, and 7 measurements of the background, vehicle, and 30 µM ENPP1 Inhibitor C Assay Reagent on the same day. The intra-assay coefficients of variation were 2, 8, and 9%, respectively. The intra-assay coefficient of variation for the IC_{50} value of eleven inhibition curves performed on the same day was 4%.

Inter-assay precision was determined by analyzing inhibition with ENPP1 Inhibitor C Assay Reagent in six separate assays on different days. The inter-assay coefficient of variance for the IC_{50} value was 11%.

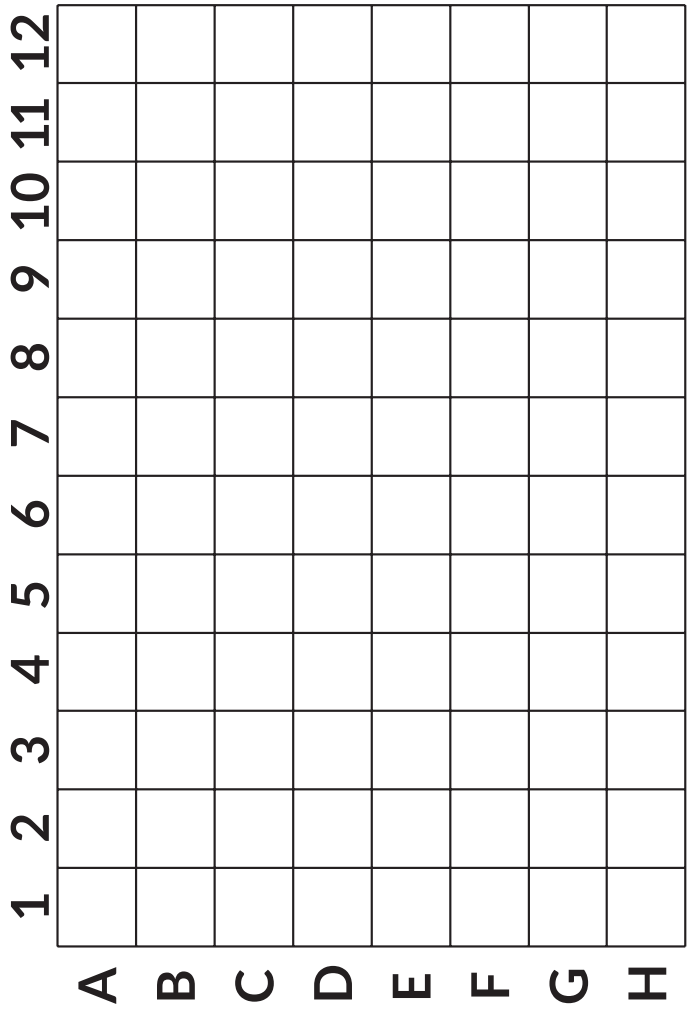
RESOURCES

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 well(s) 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 *gain* setting is too high	Reduce the *gain* 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

References

1. Onyedibe, K.I., Wang, M., and Sintim, H.O. ENPP1, an old enzyme with new functions, and small molecule inhibitors - A STING in the tale of ENPP1. *Molecules* **24(22)**, E4192 (2019).
2. Sortica, D.A., Crispim, D., Zaffari, G.P., *et al.* The role of ecto-nucleotide pyrophosphatase/phosphodiesterase 1 in diabetic nephropathy. *Arq. Bras. Endocrinol. Metab.* **55(9)**, 677-685 (2011).
3. Li, L., Yin, Q., Kuss, P., *et al.* Hydrolysis of 2'3'-cGAMP by ENPP1 and design of nonhydrolyzable analogs. *Nat. Chem. Biol.* **10(12)**, 1043-1048 (2014).
4. Bowman, M.A.H. and McNally, E.M. Genetic pathways of vascular calcification. *Trends Cardiovasc. Med.* **22(4)**, 93-98 (2012).
5. Bacci, S., De Cosmo, S., Prudente, S., *et al. ENPP1* gene, insulin resistance and related clinical outcomes. *Curr. Opin. Clin. Nutr. Metab. Care* **10(4)**, 403-409 (2007).
6. Zhang, J.-H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Scree.* **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.