Prostaglandin E_2 TR-FRET Assay Kit

Item No. 702200

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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400577	Prostaglandin E_2 Antibody Chelate	1 vial	-20°C
400578	Prostaglandin E_2 Tracer (Red)	1 vial	-20°C
400576	Prostaglandin E_2 Standard	1 vial/100 µl	-20°C
600503	TR-FRET Assay Buffer (10X)	1 vial/2 ml	-20°C
600504	TR-FRET Assay Buffer Additive	1 ea/200 mg	-20°C
600854	384-Well Solid Plate (low volume, white)	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 capable of TR-FRET with an excitation wavelength of 340 nm and emission wavelengths of 615 nm and 665 nm
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 or dilution plates
5. Timer

INTRODUCTION

Background

Prostaglandin E_2 (PGE_2) is an eicosanoid formed from arachidonic acid by COX enzymes and PGE_2 synthase (PGES) *via* PGG_2 and PGH_2 intermediates.[1] It can be produced *de novo* in all cells following cell activation and the release of arachidonic acid from plasma membrane phospholipids or when exogenous free arachidonate is available.[2] PGE_2 acts in an autocrine or paracrine fashion to bind to its receptors, EP_1, EP_2, EP_3, and EP_4, which have differing tissue distribution and cellular localizations, to initiate signaling through various pathways, including the protein kinase A (PKA), β-catenin, EGF, MAPK, NF-κB, and PI3K/Akt pathways.[1-4] Due to this, PGE_2 is involved in a wide variety of biological processes, including inflammation, fertility and parturition, gastric mucosal integrity and motility, and immune modulation in and outside of cancer.[1,2,4-8]

About This Assay

Cayman's Prostaglandin E_2 TR-FRET Assay Kit provides a robust and easy-to-use time-resolved Förster resonance energy transfer (TR-FRET) platform for measurement of PGE_2 in cell culture supernatants. The assay utilizes a PGE_2 monoclonal antibody directly labeled with a europium (Eu^{3+}) chelate as a donor molecule and fluorescently labeled PGE_2 as the acceptor molecule. In the absence of free PGE_2, the acceptor-labeled PGE_2 is bound to the europium-labeled PGE_2 antibody resulting in FRET from the donor to the acceptor upon excitation of the Eu^{3+} chelate at 340 nm and an emission from the acceptor at 665 nm. If free PGE_2 is present, it will compete with the PGE_2 acceptor for binding to the donor-labeled PGE_2 antibody, causing a decrease in the TR-FRET signal. The specific signal at 665 nm will be inversely proportional to the PGE_2 concentration in the sample. Data can be expressed as either the signal at 665 nm or the 665/615 nm ratio. This assay has a range of 0.038-600 ng/ml with lower limit of detection of approximately 0.16 ng/ml.

Introduction to TR-FRET

TR-FRET is based upon the principles of FRET but possesses a number of advantages that make it a superior technology for high-throughput screening. When an optically active molecule absorbs a photon, it has several options by which it may release that energy: it may release a photon of a longer wavelength (less energy) than the photon it absorbed, it may dissipate the energy as heat, or it can transfer the energy non-radiometrically to a suitable acceptor fluorophore. The latter effect is known as FRET, and it is a commonly used phenomenon in biological assays. In these assays, a donor flurophore is coupled to one binding partner and an acceptor fluorophore is coupled to the other binding partner. The binding partners are mixed in an assay well and allowed to associate. The donor fluorophore is then excited with a wavelength of light that does not excite the acceptor fluorophore, and if the molecules are within approximately 100 Å of each other, the donor fluorophore can non-radiometrically transfer the energy to the acceptor fluorophore, which will then release that photon as light at a wavelength characteristic of the acceptor fluorophore (see Figure 1 on page 9). For each assay point, the fluorescence intensity of the donor fluorophore and the acceptor fluorophore are measured, and the data are generally presented as the ratio of acceptor fluorophore intensity/donor fluorophore intensity. This methodology is particularly sensitive because the FRET efficiency decays as a function of the inverse 6^{th} power of the distance between the two fluorophores. Therefore, unassociated binding partners are unlikely to lie within the distance required for efficient FRET.

TR-FRET is an extension of FRET that utilizes a donor fluorophore with a long fluorescent half-life. These fluorophores are based upon lanthanide (most often Eu^{3+} or Tb^{3+}) chelates that have characteristically large Stokes shifts and fluorescent half-lives on the order of milliseconds. The long fluorescent lifetime allows the TR-FRET signal to be sustained for dramatically longer periods of time than standard fluorescence. This is particularly advantageous because it affords the ability to measure the TR-FRET signal after background fluorescence in the assay (*e.g.*, buffer/reagent autofluorescence) has dissipated. The increased signal:noise ratio and the diminished effects of screening compound fluorescence makes TR-FRET assays particularly useful for high-throughput screening applications.

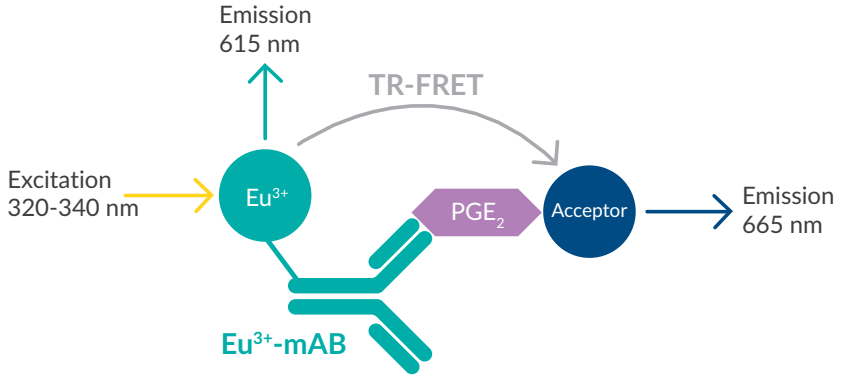

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Sample Preparation

This assay has been demonstrated to work with mammalian cell supernatant without causing any interference in the assay.

Reagent Preparation

1. **TR-FRET Assay Buffer (1X)**

   Mix 2 ml of TR-FRET Assay Buffer (10X) (Item No. 600503) with 18 ml of ultrapure water to make 20 ml of TR-FRET Assay Buffer (1X). Add TR-FRET Assay Buffer Additive (Item No. 600504) and allow it to dissolve. For best results, filter the TR-FRET Assay Buffer (1X) with a 0.22 µm filter before use. Store the diluted buffer at 4°C where it will be stable for approximately one month.

2. **Prostaglandin E_2 Antibody Chelate**

   This vial contains lyophilized Prostaglandin E_2 Antibody Chelate (Item No. 400577). Immediately prior to performing the assay, reconstitute the entire contents of the vial with 3 ml of TR-FRET Assay Buffer (1X) and mix gently. The reconstituted chelate will be stable for three hours at room temperature and for 2 weeks if stored at 4°C. If all of the Prostaglandin E_2 Antibody Chelate will not be used at one time, aliquot the reconstituted chelate and store at -20°C where it will be stable for at least six months. Avoid repeated freeze thaw cycles.

3. **Prostaglandin E_2 Tracer (Red)**

   This vial contains lyophilized Prostaglandin E_2 Tracer (Red) (Item No. 400578). Immediately prior to performing the assay, reconstitute the entire contents of the vial with 3 ml of TR-FRET Assay Buffer (1X) and mix gently. The reconstituted tracer will be stable for three hours at room temperature and for 2 weeks if stored at 4°C. If all of the Prostaglandin E_2 Tracer (Red) will not be used at one time, aliquot the reconstituted tracer and store at -20°C where it will be stable for at least six months. Avoid repeated freeze thaw cycles.

TR-FRET Plate Reader Settings

We recommend reading the TR-FRET assay at two wavelengths, detecting both the emission from the Eu^{3+} chelate donor at 615 nm and from the acceptor fluorophore at 665 nm. Table 1, below, provides instrument settings to be used as guidelines.

	TR-FRET-Compatible Plate Reader	
Parameter	**Flash Lamp Excitation**	**Laser Excitation**
Excitation Filter	320 nm (or 340 nm)	--
Emission Filters	615 nm (or 620 nm) and 665 nm (or 670 nm)	615 nm (or 620 nm) and 665 nm (or 670 nm)
Delay Time	90 µs	50 µs
Flash Energy Level	100% or High	100%
Number of Flashes	100	20
Window (Integration Time)	300 µs	100 µs

Table 1. Recommended TR-FRET plate reader settings

Preparation of Assay-Specific Reagents

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Prostaglandin E_2 Standard (Item No. 400576) several times. Using the equilibrated pipette tip, transfer 5 µl of the standard into a clean test tube, then dilute with 245 µl of TR-FRET Assay Buffer (1X). The concentration of this solution (the bulk standard) will be 6 µg/ml.

NOTE: If assaying cell supernatant samples that have not been diluted with TR-FRET Assay Buffer (1X), cell culture medium should be used in place of TR-FRET Assay Buffer (1X) for dilution of the standard curve.

Obtain eight 1.5 ml tubes and label them #1-8. Aliquot 270 µl TR-FRET Buffer (1X) to tube #1 and 240 µl of TR-FRET Assay Buffer (1X) to tubes #2-7. Transfer 30 µl of the bulk standard (6 µg/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 60 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 60 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-7. Do not add any standard to tube 8. It is the zero standard and represents maximum TR-FRET signal. These diluted standards should not be stored for more five hours at room temperature.

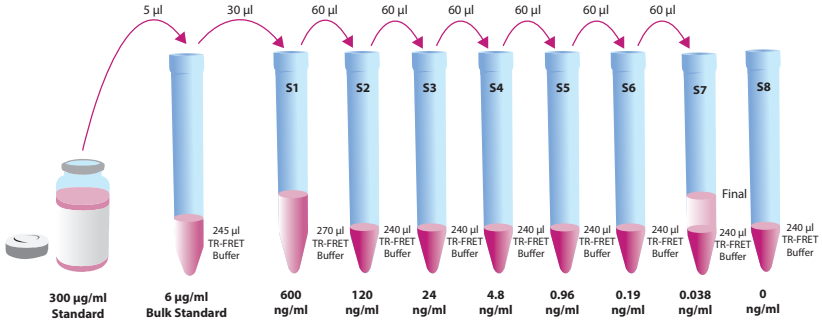

Figure 2. Preparation of the PGE_2 standards

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 two wells designated as background and an eight-point standard curve ran in duplicate. *NOTE: Each assay must contain this minimum configuration to ensure accurate and reproducible results. Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate.* A typical layout of samples to be measured in duplicate is shown in Figure 3, 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	B	B	8	8	24	24	40	40	56	56	72	72	88	88	104	104	120	120	136	136	152	152	168	168
B	S1	S1	9	9	25	25	41	41	57	57	73	73	89	89	105	105	121	121	137	137	153	153	169	169
C	S2	S2	10	10	26	26	42	42	58	58	74	74	90	90	106	106	122	122	138	138	154	154	170	170
D	S3	S3	11	11	27	27	43	43	59	59	75	75	91	91	107	107	123	123	139	139	155	155	171	171
E	S4	S4	12	12	28	28	44	44	60	60	76	76	92	92	108	108	124	124	140	140	156	156	172	172
F	S5	S5	13	13	29	29	45	45	61	61	77	77	93	93	109	109	125	125	141	141	157	157	173	173
G	S6	S6	14	14	30	30	46	46	62	62	78	78	94	94	110	110	126	126	142	142	158	158	174	174
H	S7	S7	15	15	31	31	47	47	63	63	79	79	95	95	111	111	127	127	143	143	159	159	175	175
I	S8	S8	16	16	32	32	48	48	64	64	80	80	96	96	112	112	128	128	144	144	160	160	176	176
J	1	1	17	17	33	33	49	49	65	65	81	81	97	97	113	113	129	129	145	145	161	161	177	177
K	2	2	18	18	34	34	50	50	66	66	82	82	98	98	114	114	130	130	146	146	162	162	178	178
L	3	3	19	19	35	35	51	51	67	67	83	83	99	99	115	115	131	131	147	147	163	163	179	179
M	4	4	20	20	36	36	52	52	68	68	84	84	100	100	116	116	132	132	148	148	164	164	180	180
N	5	5	21	21	37	37	53	53	69	69	85	85	101	101	117	117	133	133	149	149	165	165	181	181
O	6	6	22	22	38	38	54	54	70	70	86	86	102	102	118	118	134	134	150	150	166	166	182	182
P	7	7	23	23	39	39	55	55	71	71	87	87	103	103	119	119	135	135	151	151	167	167	183	183

B = Background Wells
S1-S8 = Standard Wells
1-183 = Sample Wells

Figure 3. 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.
- Use different tips to pipette each reagent.
- 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.
- Avoid introducing bubbles into the wells.

General Information

- The final volume of the assay is 20 µl in all the wells.
- Use the diluted assay buffer in the assay.
- All reagents should be prepared as described above and 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 emission wavelengths of 615 nm and 665 nm.

Performing the Assay

1. **Background Wells**: add 15 µl of TR-FRET Assay Buffer (1X) to three wells.
2. **Standard Wells**: add 10 µl from tube #8 to each of the zero standard wells (S8). Add 10 µl from tube #7 to each of the lowest standard wells (S7). Continue with this procedure until all the standards are aliquoted.
3. **Sample Wells**: add 10 µl of samples to the designated wells.
4. Add 5 µl of the reconstituted Prostaglandin E_2 Tracer to all wells except the background wells.
5. Initiate the reactions by adding 5 µl of reconstituted Prostaglandin E_2 Chelate to all the wells being used. Mixing the contents is not necessary.
6. Cover the plate with the Foil Plate Cover (Item No. 400023) and incubate for two hours at room temperature.
7. Gently remove the plate cover. Read on a TR-FRET-compatible plate reader. *NOTE: The same plate can be read several times without a negative effect on the assay performance, and the signal is stable for at least 5 hours at room temperature or overnight at 4°C as long as the plate is sealed to prevent evaporation.*

ANALYSIS

Calculations

1. Calculate the TR-FRET ratio for each standard and sample. Alternatively, the signal at 665 nm can be used directly to analyze the data. TR-FRET data are typically calculated and presented ratiometrically using the fluorescence intensities (FI) at 665 nm and 615 nm according to the following formula:

$$[(FI_{665\ nm}/FI_{615\ nm}) \times 1{,}000]$$

2. Plot the TR-FRET ratios of standards S1-S8 *versus* PGE_2 concentration with the y-axis and x-axis set to linear and log scales, respectively. Perform a four-parameter logistic analysis to fit the data.
3. To determine the concentration of PGE_2 in samples, use the standard curve to interpolate the corresponding [PGE_2] based on TR-FRET ratio obtained in the assay.

Software such as GraphPad Prism™ or MathLab be used to interpolate sample concentrations.

The data presented here is an example 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.

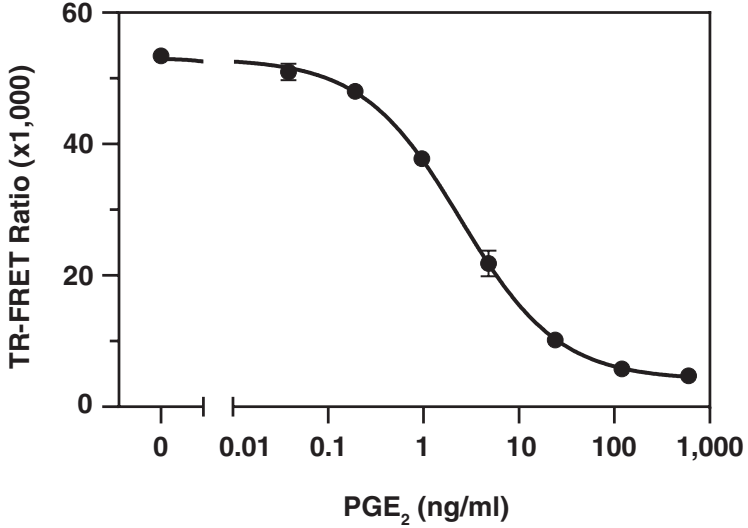

Figure 4. Typical standard curve. TR-FRET ratios are plotted as the mean of duplicate measurements ± the standard deviation *versus* the concentration of the Prostaglandin E_2 Standard ranging from 0.038 ng/ml to 600 ng/ml.

Z´ Factor:

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[9]

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

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 Prostaglandin E_2 TR-FRET Assay Kit was determined to be 0.88.

Performance Characteristics

Sample Data:

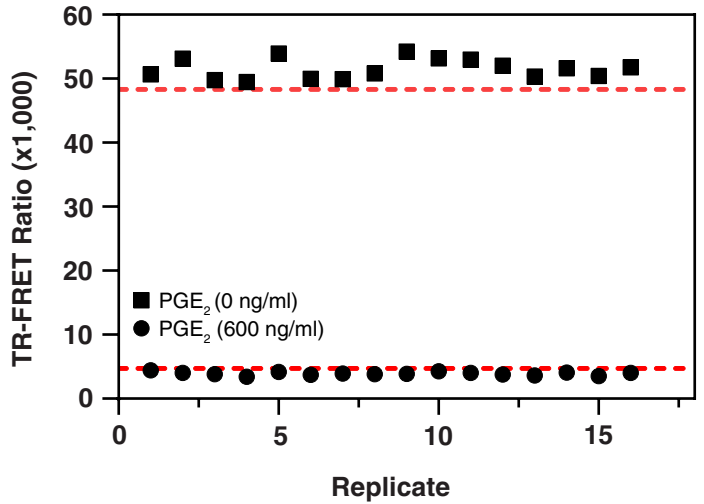

Figure 5. Typical performance data for the Prostaglandin E_2 TR-FRET Assay Kit. Data are shown from 16 replicates each for the 0 ng/ml Prostaglandin E_2 Standard (S8) and 600 ng/ml Prostaglandin E_2 Standard (S1) prepared as described in the kit booklet. The calculated Z' factor for this experiment was 0.88. The red lines correspond to three standard deviations from the mean for each control value.

Spike and Recovery:

RAW 264.7 cell supernatants were spiked with different amounts of PGE_2 and evaluated using the Prostaglandin E_2 TR-FRET Assay Kit. The results are shown below. The error bars represent the standard deviation obtained from multiple dilutions of each sample.

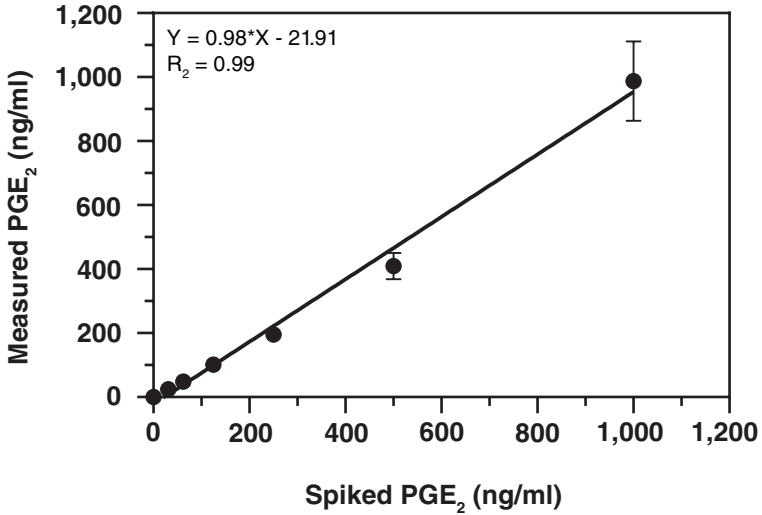

Figure 6. Spike and recovery of PGE_2 in cell supernatant

Linearity:

Stimulated RAW 264.7 cell supernatants were treated with various levels of inhibitor and evaluated for linearity using the Prostaglandin E_2 TR-FRET Assay Kit. The results are shown below.

Dilution Factor	Measured PGE_2 Concentration (ng/ml)	Linearity
1	0.84	100
2	0.98	117
1	8.04	100
2	7.56	94
4	8.02	100
2	28.09	100
4	27.75	99
2	33.39	100
4	34.90	105

Table 2. Linearity in cell supernatants

Precision:

Intra-assay precision was determined by analyzing 24 replicates of two cell supernatant controls in a single assay.

Measured PGE_2 (ng/ml)	%CV
3.7	11
867.4	7.7

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of two cell supernatant controls in five separate assays on three different days.

Measured PGE_2 (ng/ml)	%CV
6.8	19
18.1	12

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
Prostaglandin E_2	100%
Prostaglandin E_2 Ethanolamide	92%
Prostaglandin E_3	41%
Prostaglandin E_1	11%
8-*iso* Prostaglandin $F_{2\alpha}$	9.3%
Sulprostane	2.2%
6-keto Prostaglandin $F_{1\alpha}$	0.5%
13,14-dihydro 15-keto Prostaglandin E_2	0.03%
Prostaglandin A_2	0.02%
Prostaglandin D_2	<0.01%

Table 5. Cross reactivity of the Prostaglandin E_2 TR-FRET Assay Kit

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Bubble in the well B. Poor pipetting/technique	A. Carefully tap the side of the plate with your finger to remove bubbles B. Be careful not to splash the contents of the wells
Assay S/B ratio is <5 for the zero standard (Max TR-FRET signal) *versus* background	A. Plate reader and/or settings not suitable for TR-FRET assays B. Use of low-quality water for reagent preparation	A. Use a filter-based instrument to read the plate(s) B. Only use ultrapure water for preparation of the reagents

Procedure	Background Wells	Standard Wells	Sample Wells
Add TR-FRET Assay Buffer (1X)	15 µl	--	--
Add Standard/Samples	--	10 µl	10 µl
Add PGE_2 Tracer (Red)	--	5 µl	5 µl
Add PGE_2 Chelate	5 µl	5 µl	5 µl
Cover with foil plate cover and incubate at room temperature for 2 hours			
Remove foil plate cover and measure TR-FRET (ex 340 nm/em 615 and 665 nm)			

Table 6. Assay summary

References

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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.