TECHNICAL DATA SHEET

ValiScreen® GPCR Cell Line

Caution: For Laboratory Use. A research product for research purposes only

human Prostanoid FP Receptor Cell Line

Product No.: ES-564-C

Lot No.: M2W-C1

Material Provided

Cells:	2 x 1 mL frozen aliquot (ES-564-CV)
Format:	~2.5 x 10^6 cells /mL in freezing medium

Product Information

Cellular Background:	1321N1
Cell Line Development:	Our proprietary bicistronic expression plasmid containing the sequence coding for the human Prostanoid FP receptor was transfected in 1321N1 cells. Geneticin-resistant clones were obtained by limit dilution and compared for receptor expression levels using a radioligand binding assay. The clone with the highest receptor expression level was selected for characterization in binding and functional assays.
DNA Sequence:	Identical to coding sequence of GenBank NM_000959.3.
Corresponding Protein Sequence:	Identical to GenBank NP_000950.1.
Receptor expression level (B_{MAX}):	Estimated to be 1.0 pmol/mg protein, using [^{3}H]$PGF_{2\alpha}$
K_D for the above radioligand:	3.1 nM
Shipping Conditions:	Shipped on dry ice. Please ensure dry ice is still present in the package upon receipt or contact customer support.
Storage Conditions:	Store in liquid nitrogen (vapor phase) immediately upon receipt.

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Quality Control

The EC_{50} for a reference agonist was determined in Calcium flux assay. A mycoplasma test was performed using MycoAlert® (Lonza) mycoplasma detection kit. We certify that these results meet our quality release criteria.

$PGF_{2\alpha}$ (EC_{50}):	1.15×10^{-8} M
Stability:	Cells were kept in continuous culture for at least 60 days and showed no decrease of receptor expression level in a saturation binding assay (stable B_{max} and K_d) and no decrease in functional response (EC_{50}, E_{max} in calcium flux assay).
Mycoplasma:	This cell line tested negative for mycoplasma.

Recommended Cell Culture Conditions

Complete Medium:	DMEM, 10% FBS, 1 mM sodium pyruvate, 0.4 mg/ml Geneticin (receptor expression selection).
Freezing Medium:	DMEM, 10% FBS, 1 mM sodium pyruvate with 10% DMSO, without selection agents.
Thawing Cells:	Using appropriate personal protective equipment, place the frozen aliquot in a 37°C water bath (do not submerge) and agitate until its content is thawed completely. Immediately remove from water bath, spray aliquot with 70% ethanol and wipe excess with a towel. Under aseptic conditions using a pipette, transfer content to 10 mL complete medium and centrifuge (150 x g, 5 min). Resuspend cell pellet in 10 mL of complete medium and transfer to an appropriate culture flask (see recommended seeding density below). Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO_2.
Recommended Seeding Density:	19 000 – 23 000 cells/cm^2
Cell Culture Protocol:	Typically, for regular cell culture maintenance, cells are grown to 80% confluence and trypsinized (0.05% trypsin / 0.5 mM EDTA in calcium and magnesium free HBSS).

Assay Procedures

We have shown for many of our GPCR cell lines that freshly thawed cells respond with the same pharmacology as cultured cells. All of our products validated in this way are available as frozen ready-to-use cells in our catalogue. This demonstrates that cells can be prepared and frozen in advance of a screening campaign simplifying assay logistics.

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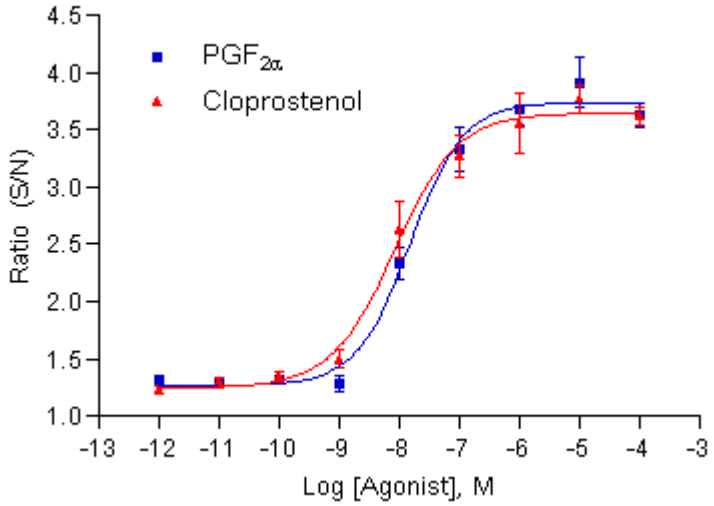
Typical Product Data – Calcium Assay (Fluorescence)



Agonist	EC_{50} (M)
$PGF_{2\alpha}$	1.5×10^{-8}
Cloprostenol	8.3×10^{-9}

Figure 1. Agonist Response in Calcium Flux assay
An agonist dose-response experiment was performed in 96-well format using 25 000 cells/well. Calcium Fluorescence was measured on FDSS6000 instrument (Hamamatsu Photonics). Data from a representative experiment are shown.

Inositol Phosphate Assay

Preliminary results showed this cell line responds positively in IP assays. Please enquire for IP accumulation assay data availability.

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Typical Product Data –Radioligand Binding Assay (Filtration)

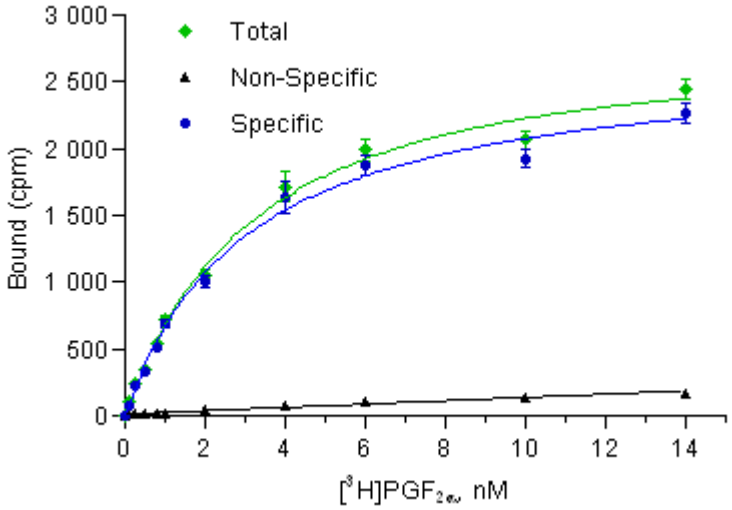
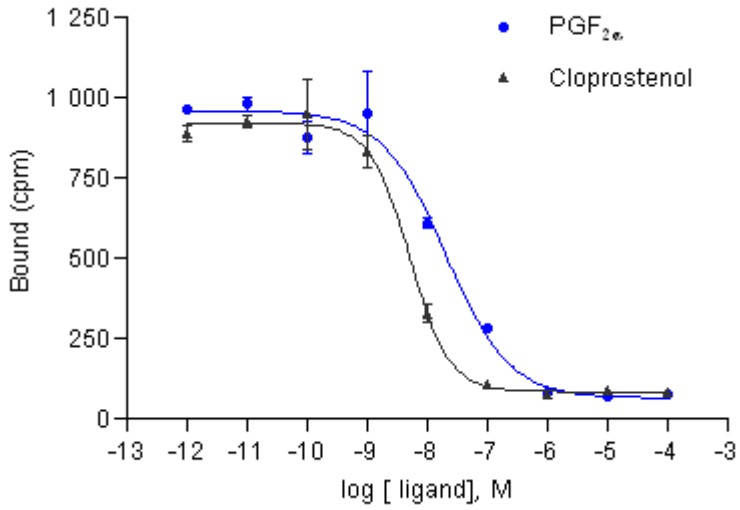


Figure 2: Saturation Binding Assay Curve (Filtration)
A saturation binding assay was performed in 96-well format using 15 µg membranes/well. Counts per minute (cpm) were measured on a TopCount® instrument. Data from a representative experiment are shown.



Agonist / Antagonist	IC_{50} (M)
$PGF_{2\alpha}$	2.0×10^{-8}
Cloprostenol	5.1×10^{-9}

Figure 3: Competition Binding Assay Curve (Filtration)
A competition binding assay was performed in 96-well format using 15 µg membranes/well. Displacement of 3 nM [^{3}H]-Prostaglandin $F_{2\alpha}$ was used. Counts per minute (cpm) were measured on a TopCount® instrument. Data from a representative experiment are shown.

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Calcium Assay Procedure (Fluorescence)

Dye solution: 5 µM Fluo-4 AM (Molecular Probes, P-6867), 1 mg/mL Pluronic acid in Assay Buffer

Assay Buffer: 2.5 mM Probenicid, 0.1% BSA, 0.05% Gelatin, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 5.6 mM Glucose, pH 7.4

Controls: Maximal Signal: 0.4% Triton X-100 (0.2% final) in Assay Buffer
Minimum signal: 0.4% Triton X-100 (0.2% final), 20 mM EGTA (10 mM final) in Assay Buffer

Reader: FDSS 6000 (Hamamatsu Photonics), Excitation 480 nm / Emission 520 to 560 nm, 96-well

Day 1		
1. Cell Culture and Harvesting:	Grow cells (mid-log phase) in culture medium without antibiotics for 18 hours, Detach gently with PBS / 0.5 mM EDTA, pH 7.4, Recover by centrifugation, Resuspend in medium without antibiotics at $2.5x10^5$ cells/mL.	
2. Cell Seeding	Distribute 100 µL (i.e. 25,000 cells) in each well of a 96 well black, clear bottom TC sterile plate, incubate overnight in a cell culture incubator (37°C, 5% CO_2).	
Day 2		
3. Cell Loading	Remove the media, and add 100 µL/well of Dye solution.	
4. Incubation	Incubate the assay plate for 1 hour at 37°C in a cell culture incubator.	
5. Ligands and compound plates preparation:	Prepare serial dilutions of 2x concentrated ligands in Assay Buffer, Dispense 100 µL/well of diluted ligand in a 96-well plate. ***Note: Assay can be miniaturized to 384-well format.***	
6. Dye Washing	Drain the media and wash the wells twice with 100 µL/well Assay Buffer,	
7. Buffer/Antagonist addition	**Agonist assay:** Add Assay Buffer to make a total of 50 µL	**Antagonist Assay:** Add 2x antagonist dilution in Assay Buffer to make a total of 50 µL
8. Equilibration	Incubate the plate for 20 min at room temperature in the dark.	
9. Plate Reading:	Using the reader's injection system, inject 50 µL per well of 2x agonist solutions in Assay Buffer, and immediately record relative light emission for 90 seconds.	Using the reader's injection system, inject 50 µL per well of 2x concentrated reference agonist in Assay Buffer (final EC_{80} concentration), and immediately record relative light emission for 90 seconds.
10. Data Analysis:	The fluorescent signal is expressed as the ratio relative to the first measurement (i.e. before dispensing), and the maximal value of this ratio during the measurement interval is used to draw sigmoidal dose-response curves.	

Important Notes:

- Probenicid is prepared as a 250 mM solution in a 50:50 mixture of 1N NaOH: Assay Buffer.

Membrane Radioligand Binding Assay Procedure (Filtration)

Note: The following are recommended assay conditions and may differ from the conditions used to generate the typical data shown in the above section.

Assay Buffer: 50 mM Tris-HCl pH 6.0, 0.5% BSA

Wash Buffer: 50 mM Tris-HCl pH 7.4

Radioligand: [^{3}H]-Prostaglandin $F_{2\alpha}$ (PerkinElmer # NET433)

Filters: Unifilter 96 GF/C (PerkinElmer # 6005174)

Membrane Binding Protocol:

Binding assays were performed in 200 µL total volume according to the following conditions. All dilutions are performed in assay buffer:

1. Membrane dilution:	µg of membranes per well, diluted in order to dispense 150µL/well. Keep on ice.
2. Assembly on ice (in 96 Deep well plate) **Saturation Binding:**	• 25 µL of assay buffer or of unlabeled ligand (Prostaglandin $F_{2\alpha}$, 3 µM final) for determination of non specific binding • 25 µL of radioligand at increasing concentrations (see figure 2) • 150 µL of diluted membranes
Competition Binding:	• 25 µL competitor ligand at increasing concentrations (see figure 3) • 25 µL of radioligand (1.96 nM final) • 150 µL of diluted membranes
3. Incubation:	60 min at 27°C.
4. Filters preparation:	GF/C filters were presoaked in 0.05 % Brij at room temperature for at least 30 min.
5. Filtration:	Aspirate and wash 9 x 500 µL with ice cold wash buffer using a FilterMate Harvester (PerkinElmer).
6. Counting:	Add 30 µL/well of MicroScint™-O (PerkinElmer # 6013611), cover filter with a TopSeal-A (PerkinElmer # 6005185) and read on a TopCount® (PerkinElmer).

References

1. Griffin BW, Williams GW, Crider JY, Sharif NA (1997) FP prostaglandin receptors mediating inositol phosphates generation and calcium mobilization in Swiss 3T3 cells: a pharmacological study. J. Pharmacol.Exp. Ther. 281:845-854.

2. Jones RL, Giembycz MA, Woodward DF (2009) Prostanoid receptor antagonists: development strategies and therapeutic applications Br J Pharmacol. 158:104-145.

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Materials and Instrumentation

The following tables provide the references of compounds and reagents used or recommended for the characterization of the human Prostanoid FP receptor ValiScreen® cell line, as well as some advice on how to use these compounds:

Table 1. References of compounds used for functional characterization and binding assays

Name	Provider	Cat n°	Working Stock Solution
Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)	Cayman	16010	10 mM in DMSO
Cloprostenol	Cayman	16765	23 mM in methanol
[^{3}H]-Prostaglandin $F_{2\alpha}$ ([^{3}H]$PGF_{2\alpha}$)	PerkinElmer	NET433	N/A

Table 2. References of cell culture media and assay buffers.

Name	Provider	Cat n°
HAM's F-12	Hyclone	SH30026.02
DMEM	Hyclone	SH30022.02
Advanced DMEM/F12 (serotonin receptors)	Invitrogen	12634-010
EMEM	BioWitthaker	06-174G
EX-CELL DHFR$^-$ media (DHFR deficient cell lines)	Sigma	C8862
FBS	Wisent	80150
FBS dialyzed	Wisent	80950
G418 (geneticin)	Wisent	400-130-IG
Zeocin	Invitrogen	R25005
Blasticidin	invitrogen	R210-01
Puromycin	Wisent	400-160-EM
Standard HBSS (with $CaCl_2$ and $MgCl_2$)	GIBCO	14025
HEPES	MP Biomedicals, LLC	101926
BSA, Protease-free	Sigma	A-3059
PEI	Sigma	P3143
Trypsin-EDTA	Hyclone	SH30236.02
Sodium Pyruvate	GIBCO	11360
L-Glutamine	GIBCO	25030
NEAA (non-essential amino acids)	GIBCO	11140
Forskolin	Sigma	F6886

Please visit our website: www.perkinelmer.com/CellLines for additional information on materials, microplates and instrumentation.

This product is not for resale or distribution except by authorized distributors.

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