TECHNICAL DATA SHEET

AequoScreen® GPCR Cell Line

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

human Somatostatin Receptor sst_4 Aequorin Cell Line

Product No.: ES-524-A

Lot No.: M12W-A2

Material Provided

Cells:	2 x 1 mL frozen aliquots (ES-524-AV)
Format:	~2.5 x 10^6 cells / mL in freezing medium

Product Information

Cellular Background:	CHO-K1
Cell Line Development:	Our proprietary bicistronic expression plasmid containing the sequence coding for the human Somatostatin receptor sst_4 was transfected in CHO-K1 cells stably expressing the mitochondrially targeted Aequorin and $G_{\alpha 16}$. Geneticin-resistant clones were obtained by limit dilution and compared for their response to a reference agonist using the AequoScreen® assay.
DNA Sequence:	Identical to coding sequence of GenBank L14856.1.
Corresponding Protein Sequence:	Identical to GenBank AAA36623.1.
Receptor expression level (B_{MAX}):	Estimated to be 11 pmol/mg protein, using [^{125}I]Somatostatin-14.
K_D for the above radioligand:	0.35 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.

PerkinElmer® For the Better

Quality Control

The EC_{50} for a reference agonist was determined in an AequoScreen® assay performed on a MicroLumat Plus (Berthold) instrument. A mycoplasma test was performed using MycoAlert® Mycoplasma (Lonza) detection kit. We certify that these results meet our quality release criteria.

Somatostatin-28 (EC_{50}):	36 nM
Stability:	Cells were kept in continuous culture for at least 60 days and showed no decrease in functional response (EC_{50}, E_{max}).
Mycoplasma:	This cell line tested negative for mycoplasma.

Recommended Cell Culture Conditions

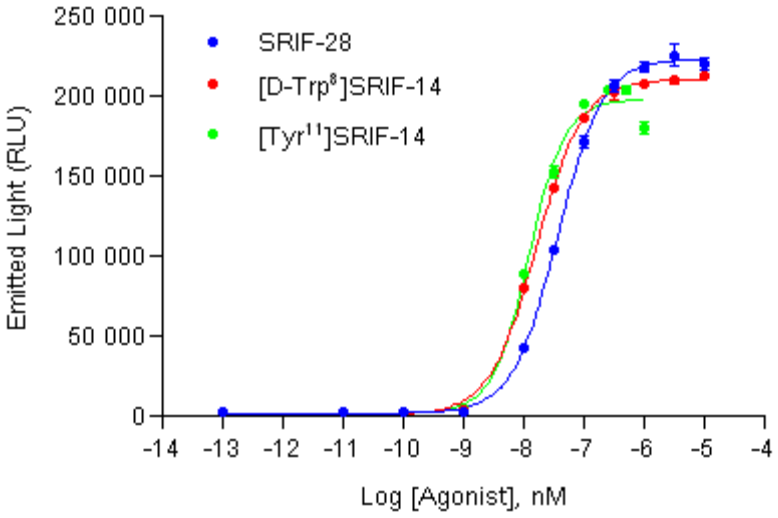
Complete Medium:	Ham's F-12, 10% FBS, 0.4 mg/mL Geneticin (receptor expression selection), 0.25 mg/mL Zeocin (Aequorin and $G_{\alpha 16}$ expression selection).
Freezing Medium:	Ham's F-12, 10% FBS with 10% DMSO, without selection agents.
Thawing Cells:	Using appropriate personal protective equipment, 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:	11 000 - 15 000 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. PerkinElmer also offers a custom service for the preparation of large quantities of frozen cryopreserved cells either from a catalogue cell line or a customer's own cell line. This demonstrates that cells can be prepared and frozen in advance of a screening campaign simplifying assay logistics.

PerkinElmer® For the Better

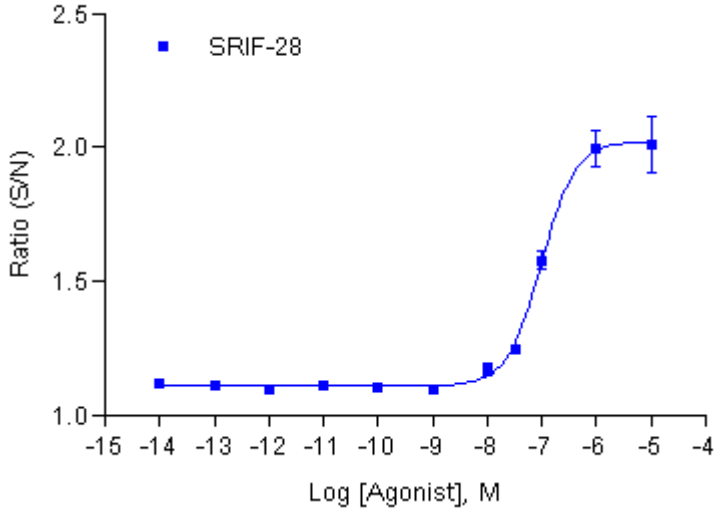
Typical Product Data – AequoScreen® Assay



Agonist	EC_{50} (M)	% of Digitonin response
Somatostatin-28 (SRIF-28)	3.6×10^{-8}	75
[D-Trp^8]SRIF-14	1.6×10^{-8}	71
[Tyr^{11}]SRIF-14	1.2×10^{-8}	67

Figure 1: Agonist Response in AequoScreen® assay
An agonist dose-response experiment was performed in 96-well format using 25 000 cells/well. Luminescence was measured on a MicroLumat Plus (Berthold). Data from a representative experiment are shown.

Typical Product Data – Calcium Assay (Fluorescence)



Agonist	EC_{50} (M)
Somatostatin-28 (SRIF-28)	9.7×10^{-8}

Figure 2. Agonist Response in Fluo-4 Calcium assay
An agonist dose-response experiment was performed in 96-well format using 25 000 cells/well. Fluorescence was measured on a FDSS6000 (Hamamatsu Photonics). Data from a representative experiment are shown.

PerkinElmer®
For the Better

Typical Product Data –Radioligand Binding Assay (Filtration)

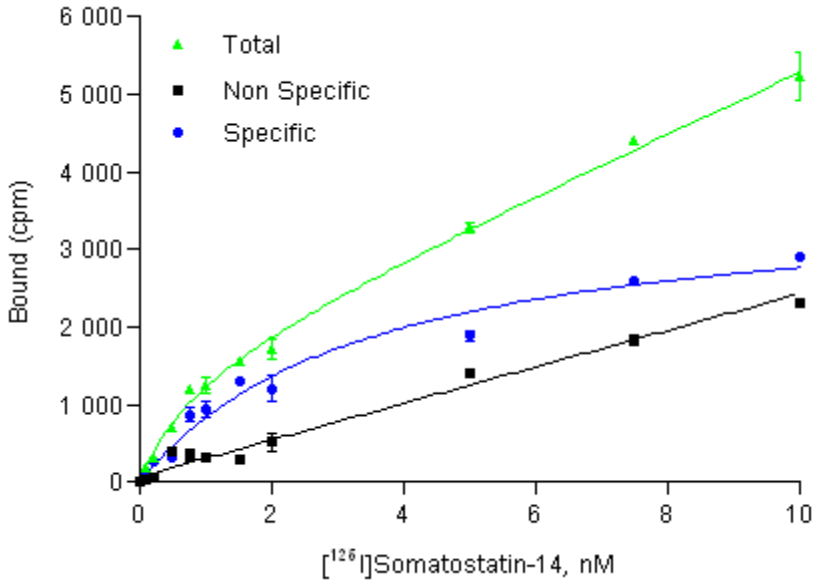


Figure 3: Saturation Binding Assay Curve (Filtration)
A saturation binding assay was performed in 96-well format using 1 µg membranes/well. Counts per minute (CPM) were measured on the TopCount®. Data from a representative experiment are shown.

PerkinElmer®
For the Better

AequoScreen® Assay Procedure (MicroBeta® JET)

Assay Buffer: DMEM / HAM's F12 with HEPES, without phenol red (Invitrogen # 11039-021) + 0.1 % protease-free BSA (from 10% solution sterilized by filtration at 0.22 µm). Store at 4°C.

Coelenterazine h: To prepare a 500 µM Coelenterazine h stock solution, solubilize 250 µg of Coelenterazine h (Promega # S2011 or Invitrogen # C6780) in 1227 µL methanol. Store at –20°C in the dark.

Digitonin: To prepare a 50 mM Digitonin stock solution, dissolve 1 g of Digitonin (Sigma # D5628) in 16.27 mL of DMSO. Aliquot and store at -20°C.

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 Assay Buffer at a concentration of $3x10^5$ cells/mL.
2. Coelenterazine Loading:	Under sterile conditions, add "Coelenterazine h" at a final concentration of 5 µM to the cell suspension, mix well. Incubate at room temperature protected from light and with constant gentle agitation for at least 4 hours (incubation can be extended overnight).
3. Cells Dilution:	Dilute cells 3x in assay buffer and incubate as described above for 60 min.
4. Ligands and plates preparation:	Prepare serial dilutions of ligands in assay buffer (2x concentration for agonists, 2x concentration for antagonists). Dispense 50 µL of diluted ligand in a 96-well Optiplate™. ***Note: Assay can be miniaturized to 384-well and 1536-well formats.***
5. Agonist Mode Reading:	Using the reader's automatic injection system, inject 50 µL of cells (i.e. 5 000 cells) per well and immediately record relative light emission for 20-40 seconds. Digitonin at a final concentration of 100 µM in assay buffer is used in control wells to measure the receptor independent cellular calcium response.
6. Antagonist Mode Reading:	After 15 minutes of incubation of the cells with the ligand, using the reader's automatic injection system, inject 50 µL of the reference agonist at a final concentration equivalent to the EC_{80} and immediately record relative light emission for 20-40 seconds.
7. Data Analysis:	Sigmoidal dose-response curves are generated using average Luminescent Counts Per Second (LCPS) recorded for 20-40 sec immediately after cells are mixed with the agonist in agonist mode or the EC_{80} of a reference agonist in antagonist mode.

Important Notes:

- Temperature should remain below 25°C during the coelenterazine loading of the cells, and until using the cells for the readings. Excessive heating by the cell stirrer for example will result in signal loss.
- Depending on (1) sensitivity of the reader used, (2) plate format used, and (3) assay characteristics wanted, it is possible to load cells at (a) different concentrations of cells and coelenterazine, (b) with different subsequent dilution factors, and (c) using different cell numbers per well. This is part of the validation work when importing an assay to a new reader.
- For tips and examples on running AequoScreen® assays on different readers, please refer to the AequoScreen® Starter Kit Manual available at www.perkinelmer.com/CellLines.

PerkinElmer®
For the Better

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 buffer A,	
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.

PerkinElmer®
For the Better

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: 25 mM HEPES pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA

Wash Buffer: 25 mM HEPES pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA (ice cold)

Radioligand: [^{125}I]Somatostatin-14 (PerkinElmer # NEX446)

Filters: Unifilter 96 GF/B (PerkinElmer # 6005177)

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:	1 µg of membranes per well, diluted in order to dispense 150 µL/well. Keep on ice.
2. Assembly on ice (in 96 Deep well plate) **Saturation Binding:**	• 25 µL of assay buffer or of unlabeled ligand (Somatostatin-28, 2 µM final) for determination of non specific binding • 25 µL of radioligand at increasing concentrations (see figure 3) • 150 µL of diluted membranes
Competition Binding:	• 25 µL competitor ligand at increasing concentrations • 25 µL of radioligand (0.4 nM final) • 150 µL of diluted membranes
3. Incubation:	60 min at 27 °C.
4. Filters preparation:	GF/B filters were presoaked in 0.5 % PEI 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).

PerkinElmer®
For the Better

References

1. Dupriez VJ, Maes K, Le Poul E, Burgeon E, Detheux M. (2002) Aequorin-based functional assays for G-protein-coupled receptors, ion channels, and tyrosine kinase receptors. Receptors Channels 8:319-30

2. Rizzuto R. Simpson AWM, Brini M, Pozzan T. (1992) Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin. Nature 358:325-327.

3. Stables J., Green A., Marshall F., Fraser N., Knight E., Sautern M., Milligan G., Lee M., Rees S. (1997) A bioluminescent assay for agonist activity at potentially any G-protein-coupled receptor. Anal. Biochem. 252:115-126.

4. Milligan G, Marshall F, and Rees S. (1996) $G_{\alpha 16}$ as a universal G protein adapter: implications for agonist screening strategies. TIPS 17:235-237.

5. Offermanns S, Simon M. (1995) $G_{\alpha 15}$ and $G_{\alpha 16}$ couple a wide variety of receptors to phospholipase C. J. Biol. Chem. 270:15175-15180.

6. Csaba Z, Dournaud P. (2001) Cellular biology of somatostatin receptors. Neuropeptides. 35:1-23.

7. Siehler S, Seuwen K, Hoyer D. (1999) Characterisation of human recombinant somatostatin receptors. 1. Radioligand binding studies. Naunyn Schmiedebergs Arch Pharmacol. 360:488-499.

8. Siehler S, Nunn C, Hannon J, Feuerbach D, Hoyer D. (2008) Pharmacological profile of somatostatin and cortistatin receptors. Mol Cell Endocrinol. 286:26-34.

PerkinElmer®
For the Better

Materials and Instrumentation

The following tables provide the references of compounds and reagents used for the characterization of the human Somatostatin Receptor sst_4 Aequorin 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
Somatostatin-28 (SRIF-28)	Bachem	H-4955	0,2 mM in PBS / 0.1% Protease-free BSA
[D-Trp^{8}]SRIF-14	Bachem	H-3198	1 mM in PBS / 0.1% Protease-free BSA
[Tyr^{11}]SRIF-14	Bachem	H-1495	0,2 mM in PBS / 0.1% Protease-free BSA
[^{125}I]Somatostatin-14	PerkinElmer	NEX446	N/A

Table 2. References of cell culture media and additives.

Note: The table below lists generic media and additives typically used for PerkinElmer cell lines. For product specific media and additives, please refer to the "Recommended Cell Culture Conditions" section.

Name	Provider	Cat n°
HAM's F-12	Hyclone	SH30026.02
DMEM	Hyclone	SH30022.02
UltraCHO (serotonin receptors)	BioWitthaker	12-724-Q
EMEM	BioWitthaker	06-174G
DHFR^{-} HAM's F-12 (for 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

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.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

Copyright ©2009, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.