human Somatostatin Receptor sst_1 Cell Line

Product No.: ES-520-C

Lot No.: 1834627

Material Provided

Cells:	2 x 1 mL frozen aliquot (ES-520-CV)
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_1 was transfected in CHO-K1 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_001049.2.
Corresponding Protein Sequence:	Identical to GenBank NP_001040.1.
Receptor expression level (B_{max}):	Estimated to be 4.3 ± 2.1 pmol/mg protein, using [^{125}I]-[Tyr^{11}]-SRIF-14
K_d for the above radioligand:	1.3 ± 0.6 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.

Quality Control

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

SRIF-28 (EC_{50}):	0.87 nM
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).
Mycoplasma:	This cell line tested negative for mycoplasma.

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.

Recommended Cell Culture Conditions (CHO-K1)

- The recommended media catalogue number and supplier reference information are listed in this Product Technical Data Sheet (last page). Media composition is specifically defined for each cell type and receptor expression selection. The use of incorrect media or component substitutions can lead to reduced cell viability, growth issues and/or altered receptor expression.
- Cells undergo major stress upon thawing, and need to adapt to their new environment which may initially affect cell adherence and growth rates. The initial recovery of the cells, and initial doubling time, will vary from laboratory to laboratory, reflecting differences in the origin of culture media and serum, and differences in methodology used within each laboratory.
- For the initial period of cell growth (i.e. until cells have reached Log-phase, typically 4-10 days), we strongly recommend removal of the antibiotics (G418, Zeocin™, Puromycin, Blasticidin, Hygromycin, Penicillin and Streptomycin) from the culture media. Immediately after thawing, cells may be more permeable to antibiotics, and a higher intracellular antibiotic concentration may result as a consequence. Antibiotics should be re-introduced when cells have recovered from the thawing stress.

Growth Medium:	Ham's F-12, 10% FBS, 0.4 mg/ml G418 (receptor expression selection).
Freezing Medium:	Ham's F-12, 10% FBS with 10% DMSO, without selection agents.

Thawing Cells: Using appropriate personal protective equipment, rapidly 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. Under aseptic conditions using a sterile pipette, transfer content to a sterile centrifuge tube containing 10 mL growth medium without antibiotics, pre-warmed at 37°C, and centrifuge (150 x g, 5 min). Discard supernatant using a sterile pipette. Resuspend cell pellet in 10 mL of pre-warmed growth medium without antibiotics by pipetting up and down to break up any clumps, and transfer to an appropriate culture flask (e.g. T-25, T-75 or T-175, 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:	Thawing:	15000 – 33000 cells/cm^2
	Log-phase:	11000 – 15000 cells/cm^2

Troubleshooting: Initial doubling time can vary between 18 and 96 hours (Average = 25 hours). If cells are still not adhering after 48 hours or grow very slowly, we recommend maintaining the cells in culture and not replacing the media before 5-6 days (cells secrete factors that can help with adherence and growth). If confluence is still <50% after 5-6 days, it is recommended that you replace the media with fresh media (without antibiotics). Do not passage the cells until they reach 80-90% confluence (Log-phase). If cells have not recovered after 10-12 days, please contact our Technical Support.

Culture Protocol: Under aseptic conditions, cells are grown to 80% confluence (Log-phase) and trypsinized (0.05% trypsin/0.5 mM EDTA in calcium and magnesium-free PBS). See recommended seeding density for Log-phase above.

Banking Protocol: Cells are grown to 70-80% confluence (Log-phase). Under aseptic conditions, remove medium and rinse the flask with an appropriate volume of calcium and magnesium-free PBS (example 10 mL for T-175). Trypsinize (0.05% trypsin/0.5 mM EDTA in calcium and magnesium-free PBS) to detach cells (example 5 mL for T-175), let stand 5-10 min at 37°C. Add fresh, room temperature growth medium (without antibiotics) to stop trypsinization and dilute EDTA (example 10 mL for T-175). Transfer cells to a sterile centrifuge tube and centrifuge (150 x g, 5 min). Discard supernatant using a sterile pipette. Resuspend cell pellet in ice-cold freezing medium by pipetting up and down to break up any clumps. Count cells and rapidly aliquot at the selected cell density (e.g. 2.5 x 10^6 cells/mL) in sterile polypropylene cryovials. Use appropriate material to ensure slow cooling (about -1°C/min) until -70°C. Transfer vials into a liquid nitrogen tank (vapor phase) for storage.

Typical Product Data –Radioligand Binding Assay (Filtration)

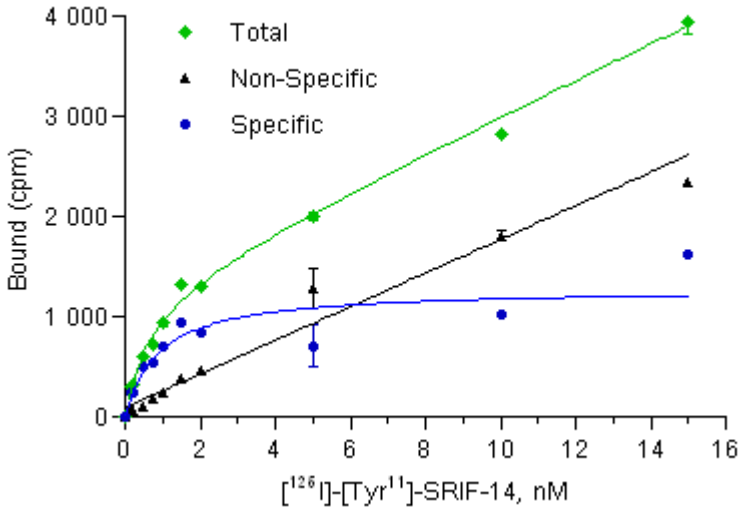
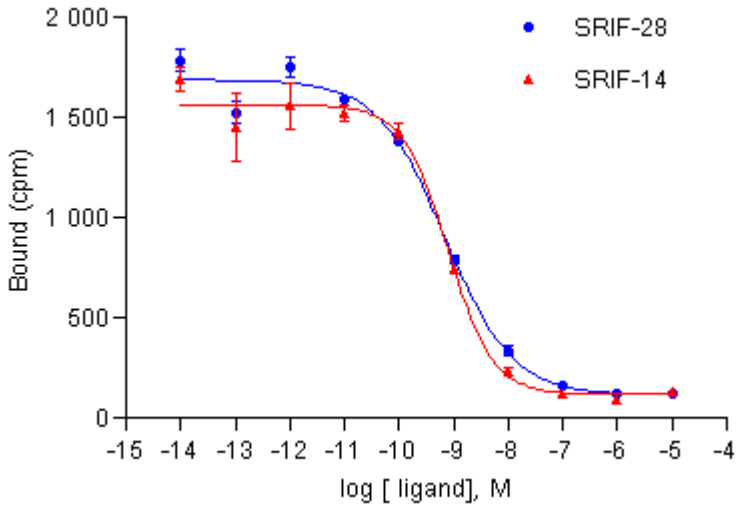


Figure 1: Saturation Binding Assay Curve (Filtration)
A saturation binding assay was performed in 96-well format using 1.5 µg membranes/well. Counts per minute (cpm) were measured on a TopCount® instrument. Data from a representative experiment are shown.



Agonist / Antagonist	IC_{50} (M)
SRIF-28	**7.0×10^{-10}**
SRIF-14	**7.8×10^{-10}**

Figure 2: Competition Binding Assay Curve (Filtration)
A competition binding assay was performed in 96-well format using 2 µg membranes/well. Displacement of 0.09 nM [^{125}I]-[Tyr^{11}]-SRIF-14 was used. Counts per minute (cpm) were measured on a TopCount® instrument. Data from a representative experiment are shown.

Typical Product Data – GTPγS - SPA® Assay

Agonist	EC_{50} (M)
SRIF 28	**1.3×10^{-9}**

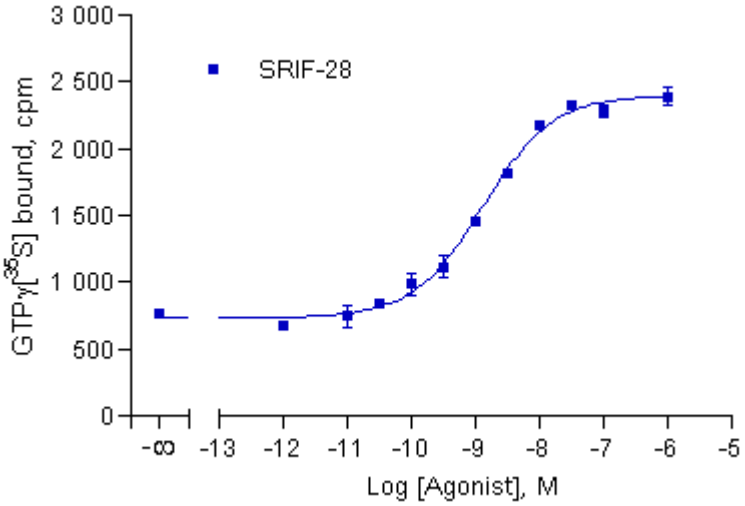


Figure 3. Agonist Response in GTPγS - SPA® assay
An agonist dose-response scintillation proximity assay (SPA) was performed in 96-well format using 5 µg membranes/well. Counts per minute (cpm) were measured on a TopCount® instrument. Data from a representative experiment are shown.

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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, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA

Wash Buffer: 50 mM Tris-HCl pH 7.4 à 4°C, 0.2% BSA

Radioligand: [^{125}I]-[Tyr^{11}]-SRIF-14 (Revvity # NEX446)

Filters: Unifilter 96 GF/C (Revvity # 6055690)

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:	2 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 (SRIF-28, 3 µM final) for determination of non specific binding • 25 µL of radioligand at increasing concentrations (see figure 1) • 150 µL of diluted membranes
Competition Binding:	• 25 µL competitor ligand at increasing concentrations (see figure 2) • 25 µL of radioligand (0.11 nM final) • 150 µL of diluted membranes
3. Incubation:	60 min at 27°C.
4. Filters preparation:	GF/C 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.
6. Counting:	Add 30 µL/well of MicroScint™-O (Revvity # 6013611), cover filter with a TopSeal-A PLUS (Revvity # 6050185) and read on a TopCount®.

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GTPγS - SPA® Assay Procedure

Assay Buffer: 20 mM HEPES pH 7.4, 100 mM NaCl, 10 µg/ml saponin, 3 mM $MgCl_2$, 0.1% protease free BSA

GDP concentration: 1 µM GDP (final)

SPA Beads: PVT-WGA (Revvity # RPNQ0001), 0.5 mg/well

Radioligand: GTPγS, [^{35}S] - (Revvity # NEG030H)

Membranes: 5 µg/well

Format: 96-well

Final volume: 100 µL/well

GTPγS-SPA assays were performed in 100 µL total volume according to the following conditions. All dilutions are performed in assay buffer:

1. Membrane Dilution:	5 µg of membranes per well, diluted in order to dispense 20 µL/well. Keep on ice.
2. GDP preparation:	Prepare a 5-fold concentrated GDP solution (i.e. 5 µM).
3. GTPγS, [^{35}S] - dilution:	Dilute GTPγS, [^{35}S] - to give ~25.000 dpm/20µL
4. Beads:	Dilute beads to 25 mg/mL (0.5 mg/20 µL)
5. Assembly (in Optiplate™), Agonist Assay:	• 20 µL of 5x GDP dilution • 20 µL of 5x agonist dilutions at increasing concentrations • 20 µL of diluted membranes
Antagonist Assay:	• 20 µL of 5x GDP dilution • 20 µL of a 5x antagonist at increasing concentrations: 5x reference agonist dilution (to reach a final concentration corresponding to its EC_{80}) • 20 µL of diluted membranes
6. Pre-incubation:	Incubate for 15 min at room temperature (RT)
7. Assemble (continued)	• 20 µL of the GTPγS, [^{35}S] - dilution • 20 µL of the SPA Beads dilution
8. Incubation:	• Cover plate with a TopSeal, • Shake on an orbital shaker for 2 min, • Incubate for 1h at RT • Centrifuge the plate for 10 min. at 2000 rpm, • Incubate for 0h to 1h at RT°
9. Counting	Count for 1 min on a TopCount®

References

1. Yamada Y, Post SR, Wang K, Tager HS, Bell GI, Seino S. (1992) Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney Proc Natl Acad Sci U S A. 89:251-255.

2. Kubota A, Yamada Y, Kagimoto S, Yasuda K, Someya Y, Ihara Y, Okamoto Y, Kozasa T, Seino S, Seino Y (1994) Multiple effector coupling of somatostatin receptor subtype SSTR1. Biochem Biophys Res Commun. 204:176-186.

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

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

Materials and Instrumentation

The following tables provide the references of compounds and reagents used or recommended for the characterization of the human Somatostatin receptor sst_1 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 no	Working Stock Solution
SRIF-28 (Somatostatin-28)	Bachem	H-4955	200 µM in PBS / 0.1% protease-free BSA
SRIF-14 (Somatostatin-14)	Bachem	H-1490	200 µM in 1% Acetic Acid
[^{125}I][Tyr^{11}]-Somatostatin-14 *	Revvity	NEX446	N/A
[^{125}I][Tyr^{11}]-Somatostatin-14 *	Revvity	NEX389	N/A

** These are the same radiolabelled molecules, but provided in different buffers. Data shown herein were generated using NEX446. Please refer to their respective Certificates of Analysis on Revvity.com for details.*

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

Name	Provider	Cat no
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

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