TECHNICAL DATA SHEET

AequoScreen® GPCR Cell Line

Research use only. Not for use in diagnostic procedures.

CHO-K1 (+$G_{\alpha16}$, medium level) Parental Cell Line

Product No.: ES-000-A2

Lot No.: M1W-A2

Material Provided

Cells:	2 x 1 mL frozen aliquot
Format:	~2.5 x 10^6 cells/mL in Ham's F12, 10% FBS with 10 % DMSO

Product Information

Cellular Background:	CHO-K1
Cell Line Development:	Our proprietary bicistronic expression plasmid containing the coding sequences of the mitochondrially targeted Aequorin and $G_{\alpha16}$ was transfected into CHO-K1 cells. Zeocin-resistant clones were obtained by limiting dilution and compared for their response to a reference agonist for endogenous Purinergic receptors using the AequoScreen® assay.
DNA Sequence:	Not applicable.
Corresponding Protein Sequence:	Not applicable.
Receptor expression level (B_{MAX}):	Not applicable.
K_D for the above radioligand:	Not applicable.
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 an AequoScreen® assay performed on MicroBeta®JET instrument. A mycoplasma test was performed using MycoAlert® Mycoplasma (Lonza) detection kit. We certify that these results meet our quality release criteria.

ATP (EC_{50}):	261 nM
Stability:	Cells were kept in continuous culture for at least 60 days and showed no decrease in functional response (EC_{50}, Emax).
Mycoplasma:	This cell line tested negative for Mycoplasma.

Recommended Cell Culture Conditions

Complete Medium:	HAM's F12, 10% FBS, and, 250 µg/mL Zeocin™.
Freezing Medium:	HAM's F12, 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.

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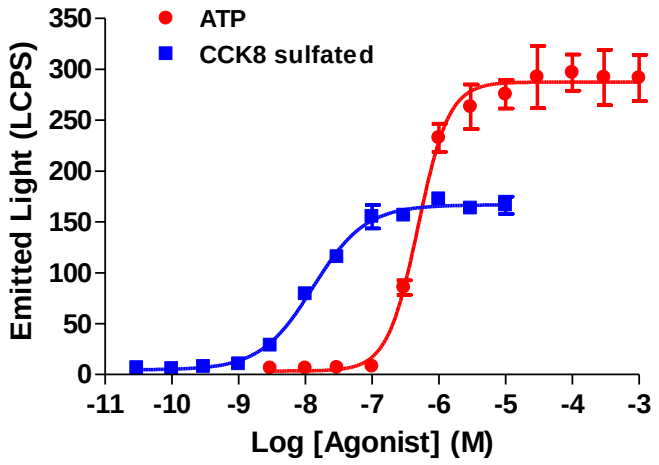
Typical Product Data AequoScreen® Assay



Agonist	EC_{50} (M)	% of Digitonin response
ATP	4.9×10^{-7}	82
CCK8 (sulfated)	1.3×10^{-8}	50

Figure 1: Agonist Response in AequoScreen® assay
An agonist dose-response experiment was performed in 96-well format using 5 000 cells/well. Luminescence was measured with MicroBeta® JET. Data from a representative experiment are shown. The Z'-factor was calculated for ATP with at least 16 background and 16 maximal signal points (Z'= 0.79).

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AequoScreen® Assay Procedure (MicroBeta® JET)

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

Coelenterazine h (500 µM): Solubilize 250 µg of Coelenterazine h (Promega # S2011 or Invitrogen # C6780) in 1227 µL methanol. Store at -20°C in the dark.

Digitonin (50 mM): 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.

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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) Gα16 as a universal G protein adapter: implications for agonist screening strategies. TIPS 17:235-237.

5. Offermanns S, Simon M. (1995) Gα15 and Gα16 couple a wide variety of receptors to phospholipase C. J. Biol. Chem. 270:15175-15180.

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Materials and Instrumentation

The following tables provide the references of compounds and reagents used for the characterization of the CHO-K1 (+$G_{\alpha 16}$, medium level) Parental Aequorin cell line, as well as some advice on how to use these compounds:

Table 1. References of compounds used for functional characterization

Name	Provider	Cat n°	Working Stock Solution
ATP (Adenosine 5′-triphosphate)	Sigma	A7699	50 mM in water
CCK8 (sulfated; ammonium salt)	Bachem	H-2080	1 mM in PBS/0.1% BSA

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.

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