

# Technical Data Certificate of Analysis

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

## AequoScreen® Double Transfected Cell Lines human Chemokine CXCR3 AequoScreen® Cell Line

PRODUCT NO.: ES-142-A  
LOT NO.: 451-494-A

### MATERIAL PROVIDED

<b>CELLS:</b>	2 x 1 ml frozen aliquots (ES-142-AV)
<b>FORMAT:</b>	~2.5x10 <sup>6</sup> cells / ml in complete medium with 10 % DMSO
<b>STORAGE CONDITIONS:</b>	Store in Liquid Nitrogen
<b>RECEPTOR CDNA:</b>	2 x 10 µl frozen pBluescript aliquots (ES-142-AC)
<b>FORMAT:</b>	1 µg / µl in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
<b>STORAGE CONDITIONS:</b>	Store at -20°C

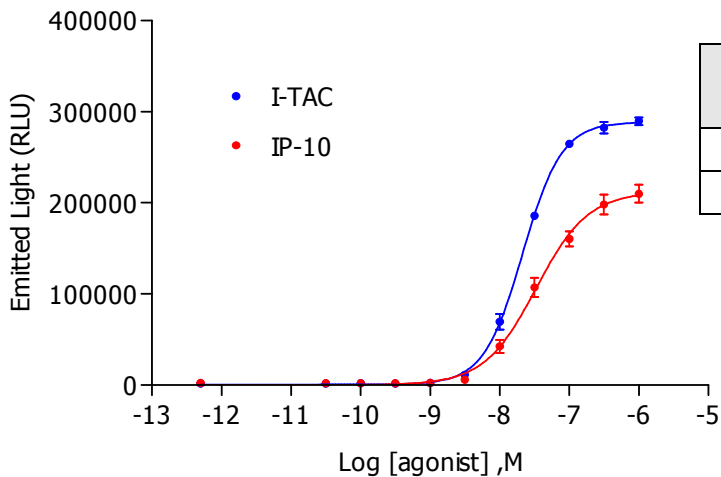
### PRODUCT INFORMATION

<b>CELLULAR BACKGROUND:</b>	CHO-K1
<b>CELL LINE DEVELOPMENT:</b>	Our proprietary bicistronic expression plasmid containing the coding sequence of the human Chemokine CXCR3 receptor was transfected, using Fugene 6, in CHO-K1 cells stably expressing the mitochondrially targetted Aequorin and G <sub>α16</sub> . Resistant clones were obtained by limit dilution and compared for their response to a reference agonist using the AequoScreen® assay.
<b>DNA ACCESSION NUMBER:</b>	Identical to coding sequence of GenBank X95876
<b>PROTEIN ACCESSION NUMBER:</b>	Identical to coding sequence of SwissProt P49682
<b>MEMBRANE RECEPTOR (B<sub>MAX</sub>):</b>	21 pmol/mg
<b>K<sub>D</sub> FOR [125I]I-TAC :</b>	0.78 nM

### QUALITY CONTROL

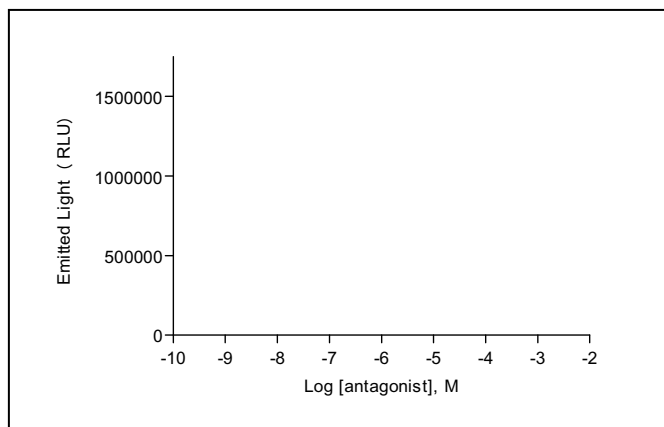
<b>REFERENCE AGONIST – I - TAC (EC<sub>50</sub>):</b>	21 nM (AEQUOSCREEN® ASSAY)
<b>STABILITY:</b>	Cells were kept in continuous culture for 90 days and showed no decrease in functional response (EC <sub>50</sub> , Emax) after 30, 60 and 90 days.
<b>MYCOPLASMA:</b>	The cell line tested negative for Mycoplasma.

## TYPICAL PRODUCT DATA



Agonist	EC <sub>50</sub> (M)	Digitonin (RLU)	% Digitonin response
I-TAC	2.1 X 10 <sup>-8</sup>	471 736	61
IP-10	3.8 X 10 <sup>-8</sup>	471 736	47

**Figure 1: Agonist Response in AequoScreen® assay**  
 96-well Agonist Dose Response curves: Concentration response curves (25 000 cells/well, Reader: MicroLumat Plus). Data from a representative experiment are shown.



**Figure 2: Antagonist Response in AequoScreen® assay**  
 Not Tested.

## RECOMMENDED CELL CULTURE CONDITIONS

**CELL CULTURE MEDIUM:** Ham's F12; 10% FBS; 100 IU/ml penicillin; 100 µg/ml streptomycin; 400 µg/ml G418 (receptor expression selection); and 250 µg/ml Zeocin™ (Aequorin expression selection).

**THAWING CELLS:** Using appropriate personal protective equipment, place the frozen ampoule in a 37°C water bath (do not submerge) and agitate until its content is thawed completely. Immediately remove from water bath, spray ampoule with 70% ethanol and wipe excess with sterile 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 table). Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>.

Cell Type	Cells/cm <sup>2</sup>
CHO	11 000 – 15 000
HEK293	41 000 – 45 000
1321N1	19 000 – 23 000

**CELL CULTURE PROTOCOL:** Typically, for regular cell culture maintenance, cells are grown to 80% confluence, trypsinized (0.05% trypsin / 1 mM EDTA in calcium and magnesium free PBS) and diluted 1/5. Under these conditions, cell passages should be carried out every 3-5 days.

## AequoScreen® ASSAY

### EXPERIMENTAL PROCEDURE (PHARMACOLOGY ASSAYS):

1. Grow cells (mid-log phase) in medium without antibiotics specify only when needed for 18 hours, detach gently with PBS – 5 mM EDTA, pH 7.4, recover by centrifugation and resuspend in assay medium (DMEM/HAM's F12 with HEPES, without phenol red + 0.1 % BSA) at a concentration of 1x10<sup>6</sup> cells/ml.
2. Add "Coelenterazine h" at a final concentration of 5 µM in assay medium; incubate at room temperature protected from light and with constant agitation for at least 4 hours (Incubation can be extended overnight).
3. Dilute cells 10 fold in assay medium and incubate as described above for 60 min.
4. Dispense 50 µl of agonist at the desired concentrations diluted in the assay medium in a 96 well Optiplate™. **Note: different microplates may be recommended depending on the instrument being used.**

*For antagonist testing, 50 µl of antagonists are dispensed at the desired concentration in the assay medium in a 96 well Optiplate™.*

5. Using the reader's automatic injection system, inject 50 µl of cells (i.e. 5 000 cells) per well in triplicate and record relative light emission for 20-40 seconds (LumiLux®, Microbeta® Jet, EnVision™, or Victor™). Digitonin at a final concentration of 50 µM diluted in assay medium is used to measure the receptor independent cellular calcium response.

*For antagonist testing, using the reader's automatic injection system, inject 50 µl of cells (i.e. 5 000 cells) per well in triplicate. After 15 minutes of incubation, using the reader's automatic injection system, inject 50 µl of the reference agonist at a final concentration equivalent to the EC<sub>80</sub>, and record relative light emission for 20-40 seconds.*

## REFERENCES

- Cox M.A. et al., *Mol. Pharmacol.*, 2001, **59**, 707-715.
- Loetscher M. et al., *Eur.J. Immunol.*, 1998, **28**, 3696-3705.
- Loetscher M. et al., *J. Exp. Med.*, 1996, **184**, 963-969.

## SUGGESTED MATERIAL AND INSTRUMENTATION

	SUPPLIER	CATALOGUE #
<b>CELL CULTURE</b>		
• HAM F-12 (CHO cells)	Invitrogen	21765
• Ultra CHO (Serotonin receptors)	Lonza	BE12-724Q
• EMEM (HEK293 cells)	Lonza	BE06-174G
• DMEM (1321N1 cells)	Lonza	12-604F
• Sodium Pyruvate (1321N1 cells)	Invitrogen	11360-039
• PEN-STREP	Lonza	DE17-602E
• G418	Invitrogen	10131-027
• Zeocin™	Invitrogen	R250-01
• Puromycin	Sigma	P7255
• PBS	Lonza	BE17-515Q
<b>AEQUOSCREEN® ASSAY</b>		
• DMEM/F-12	Invitrogen	11039
• BSA, protease free	Serva	11926
• Coelenterazine h	Promega	S2011
	Invitrogen	C6780
• Digitonin	Sigma	37006
• I-TAC	R&D Systems	672-IT
<b>MICROPLATES</b>		
• 96 well Optiplat™ (for use with the Microbeta® Jet, Envision™ and Victor™)	<b>PerkinElmer</b>	<b>6005290</b>
• 384 well Optiplat™ (for use with the Envision™ and Victor™)	<b>PerkinElmer</b>	<b>6007290</b>
• 384 well black clear bottom microplate (for use with the LumiLux®)	Greiner	781096
• 1536 well black clear bottom microplate (for use with the LumiLux®)	Greiner	783096
<b>INSTRUMENTS (visit the web site or contact your local sales office for more information)</b>		
• Microbeta® Jet	<b>PerkinElmer</b>	<b>1450-024 + 1450-221</b>
• Envision™ with Injectors	<b>PerkinElmer</b>	<b>2103-0020 + 2203-1060</b>
• Victor™ with Injectors	<b>PerkinElmer</b>	<b>1420-060 / 1420-032 + 1420-2550</b>
• LumiLux®-384 (110-120V, 60 Hz)	<b>PerkinElmer</b>	<b>PTSSPL11</b>
• LumiLux®-384 (220V, 50 Hz)	<b>PerkinElmer</b>	<b>PTSSPL12</b>
• LumiLux®-1536 (115V, 60 Hz)	<b>PerkinElmer</b>	<b>PTSSPL21</b>
• LumiLux®-1536 (230V, 50 Hz)	<b>PerkinElmer</b>	<b>PTSSPL22</b>

\*The PerkinElmer logo and design, AequoScreen, LumiLux and Microbeta are registered trademarks of PerkinElmer, Inc. Optiplate, EnVision, Microbeta, and Victor are trademarks of PerkinElmer, Inc. or its subsidiaries, in the United States and other countries. All other trademarks not owned by PerkinElmer, Inc. or its subsidiaries that are depicted herein are the property of their respective owners.