TECHNICAL DATA SHEET

cAMPZen®

Human Cannabinoid CB_1 Receptor, Frozen Cells

Product No.: ES-110-CF

Lot No.: 2431783

Material Provided

Cells:	1 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
Frozen cells info:	Frozen recombinant, CHO-K1 cells expressing the Human Cannabinoid CB_1 receptor.
DNA Sequence:	Identical to coding sequence of GenBank NM_001160260.1.
Corresponding Protein Sequence:	Identical to Swiss-Prot P21554.1
Storage Conditions:	Store in liquid nitrogen (vapor phase) immediately upon receipt, or maximum 15 days at -80°C. cAMPZen® is designed for single use only. Do not refreeze.

Quality Control

EC_{50} for a reference agonist is determined using a LANCE® cAMP assay (Figure 1). Mycoplasma test is performed using MycoAlert® Mycoplasma detection kit. We certify that these results meet our quality release criteria.

(R)-(+)-WIN 55,212-2 - (EC_{50}):	73 nM
Mycoplasma:	This cell line tested negative for Mycoplasma.

Recommended Thawing Conditions and Handling of Frozen Cells

- Carefully follow instructions below to obtain the expected results. Most Frozen cells are intended to be assayed immediately upon thawing. Exceptionally, where specified, some frozen cell products require an overnight incubation in Cell Medium to enable them to perform optimally.

- 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. The use of incorrect media or component substitutions can lead to altered product performance. Additionally, the instructions for the preparation of ligands must be carefully followed to avoid ligand precipitation, degradation or adsorption. Inappropriate preparation may result in a non-representative pharmacology.

- The complete thawing procedure must not exceed 30 min. Cell viability below 90% upon thawing may indicate that the Frozen cells were affected by incorrect thawing procedure and may yield to lower performance. Ensure the cells are not clumped and are evenly distributed in the assay plates. Gently pipet up and down if cells are clumped before dispensing the cells. Frozen cells cannot be re-frozen.

Assay Medium (for immediate thaw and use): LANCE kit Assay Buffer (see below)

Cell Medium (for overnight incubation prior to use): Ham's F-12, 10% FBS

Thawing Cells:

- Using appropriate personal protective equipment, rapidly place the frozen aliquot in a 37°C water bath (do not submerge) 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, gently resuspend the cells in the cryovials and transfer content to a sterile centrifuge tube containing 10 mL of the Assay or Cell Medium pre-warmed to 37°C, and centrifuge (150 x g, 5 min.). Do not exceed the recommended centrifugal force. Discard supernatant using a sterile pipette. Gently resuspend cell pellet in 5 mL of appropriate pre-warmed medium by gently pipetting up and down to break up any clumps. For immediate use, dilute cells to recommended cell density in Assay Medium.

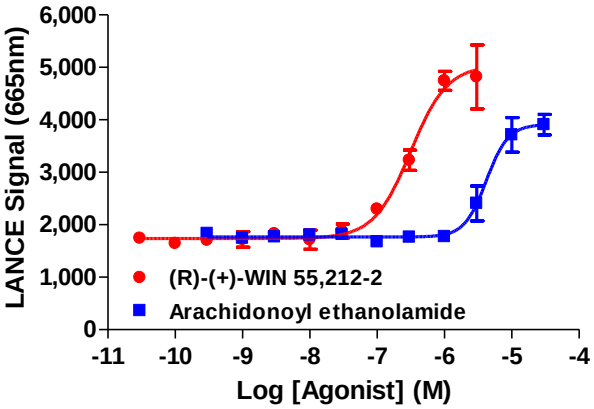
- For an overnight incubation step, plate the cells in Cell Medium in a T25 cm^2 culture flask. Incubate overnight at 37°C in a humidified atmosphere with 5% CO_2. To harvest cells, under aseptic conditions, remove media, rinse with 1.5 mL of calcium and magnesium-free PBS, add 1.5 mL Versene or calcium and magnesium-free PBS/0.5 mM EDTA, and incubate at room temperature until cells detach (do not exceed 5-10 minutes). Add 3 mL of Assay Medium, collect the cells, centrifuge (150 x g, 5 min) and resuspend in Assay Medium to the recommended cell density.

Recommended Cell Density per Assay Point (LANCE®): 2 500 cells/well

- Do not dilute the cells below the recommended cell density. Cell density per assay point will depend on the kit used to determine cAMP concentration. As a general rule, 4 to 5 times less cells are used when working with the cAMP LANCE® *Ultra* compared to when working with the cAMP LANCE® kit. The optimal cell density when using other cAMP kits needs to be determined.

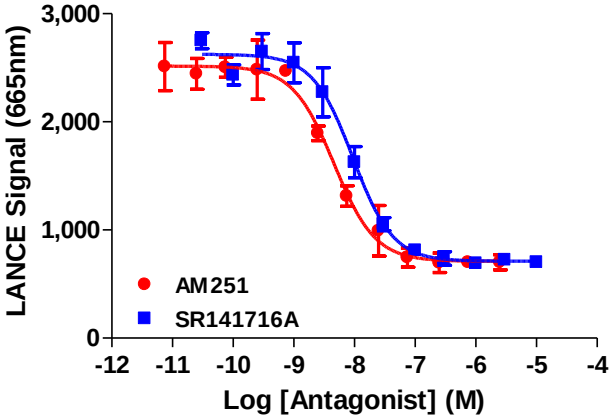
- Ligand(s) and cells must be well mixed. When running a cAMP assay, centrifuging the plate (150 x g, 30 sec.) after addition of cells and ligands will ensure adequate mixing. If this step is omitted, the cells may not respond to the ligands as expected because insufficient contact with the ligand was made.

Typical Product Data



Agonist	EC_{50} (M)
(R)-(+)-WIN 55,212-2	3.1×10^{-7}
Arachidonoyl ethanolamide	4.2×10^{-6}

Figure 1: Agonist Response in LANCE® *Ultra* cAMP assay
An agonist dose-response experiment was performed in 384-well format using 2 500 cells/well. Cell stimulation was performed for 30 min at room temperature with 10 µM Forskolin (FK) and the indicated agonist concentrations. Reader: EnVision® (laser mode). Data from a representative experiment are shown. The Z'-factor was calculated for (R)-(+)-WIN 55,212-2 with at least 16 background and 16 maximal signal points (Z'= 0.53).



Antagonist	IC_{50} (M)
AM251	4.6×10^{-9}
SR141716A	9.5×10^{-9}

Figure 2: Antagonist Response in LANCE® *Ultra* cAMP assay
An antagonist dose-response experiment was performed in 384-well format using 2 500 cells/well and 525 nM (R)-(+)-WIN 55,212-2. Cell stimulation was performed for 30 min at room temperature, and the agonists, antagonist and 10 µM Forskolin were added simultaneously. Reader: EnVision® (laser mode). Data from a representative experiment are shown.

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LANCE® *Ultra* cAMP Assay Procedure

Stimulation Buffer: HBSS, 5 mM HEPES, 0.1 % Protease-free BSA, 0.5 mM IBMX, pH 7.4.

Cells/well: For compounds not tested herein we recommend titrating the cells for optimal performance, i.e. 500-3 000 cells per assay point.

cAMP measurements can be performed with the LANCE® *Ultra* cAMP 384 Kit (Revvity # TRF0262), according to the manufacturer instructions. Briefly:

Protocols for a 384-well white Optiplate (total assay volume of 20 µL):

cAMP Standard curve	G_s Agonist	G_s Antagonist	G_i Forskolin titration	G_i Agonist	G_i Antagonist
5 µL cAMP Standard	5 µL cell suspension	5 µL cell suspension	5 µL cell suspension	5 µL cell suspension	5 µL cell suspension
5 µL Stimulation Buffer	5 µL Agonist	2.5 µL Antagonist	5 µL Forskolin	2.5 µL Agonist	2.5 µL Antagonist
-	-	2.5 µL Agonist	-	2.5 µL Forskolin	2.5 µL Forskolin/Agonist
Incubate 30 min at room temperature (optional step for cAMP Standard curve)					
5 µL 4X Eu-cAMP Tracer Working Solution					
5 µL 4X U*Light*-anti-cAMP Working Solution					
Incubate 1 h at room temperature					
Read on an EnVision® instrument. Remove microplate seal prior to reading					

1. Thawed cells prepared as described above are resuspended in stimulation buffer at the desired concentration cells/mL.

2. Prepare the 4X Tracer Working Solution by making a 1/50 dilution of the Eu-cAMP stock solution in the cAMP Detection Buffer.

3. Prepare an U*Light*-anti-cAMP Intermediate Solution by making a 1/10 dilution of the U*Light*-anti-cAMP stock solution in cAMP Detection Buffer. Prepare the 4X U*Light*-anti-cAMP Working Solution by making a 1/30 dilution of the U*Light*-anti-cAMP intermediate solution in the cAMP Detection Buffer.

Notes:

For 96- and 1536-well formats, adjust proportionally the volume of each assay component in order to maintain the volume ratios for the 384-well format. Do not modify the Eu-cAMP and/or the U*Light*-anti-cAMP concentrations.

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LANCE® cAMP Assay Procedure

Precautions and Recommendations:

- Do not vigorously vortex solutions containing cAMP antibody.
- When preparing the Detection Mix, always dilute the Eu-SA component first, and then add the Biotin-cAMP component to the Eu-SA solution.

Assay Buffer: HBSS, 5 mM HEPES, 0.1 % Protease-free BSA, 0.5 mM IBMX, pH 7.4.

Cells/well: For compounds not tested herein we recommend titrating the cells for optimal performance, i.e. 1000-10 000 cells per assay point.

Antagonist Pre-incubation: Simultaneous addition of antagonists with reference agonist.

Agonist Stimulation: 30 min at room temperature (22°C).

cAMP measurements were performed with the LANCE® cAMP 384 Kit (Revvity # AD0263), according to the manufacturer instructions. Briefly:

1. Compounds (6 µL/well) were dispensed into a 384-well white Optiplate:

	$G\alpha_s$ and $G\alpha_i$ assay modes		$G\alpha_s$ assay mode		$G\alpha_i$ assay mode	
	Basal	Forskolin	Agonist Assay	Antagonist Assay	Agonist Assay	Antagonist Assay
Buffer	6 µL	-	-	-	-	-
Antagonist	-	-	-	3 µL of 4x final conc.	-	3 µL of 4x final conc.
Agonist	-	-	6 µL of 2x final conc.	3 µL of 4x final conc.	6 µL of 2x final conc. in 2x final FK conc.	3 µL of 4x final conc. in 4x final FK conc.
Forskolin	-	6 µL of 2x final conc.	-	-		

2. Thawed cells prepared as exposed above were resuspended in assay buffer at the concentration of 8.3 x 10^5 cells/mL.
3. The Alexa Fluor® 647-anti cAMP antibody was added 1/100 (vol/vol) to the cells suspension.
4. 6 µL/well of cell and antibody suspension (5 000 cells/well) were dispensed on top of the compounds prepared in the 384 well Optiplate.
5. After incubation for 30 min at room temperature the reaction was stopped by addition of 12 µL of Detection Mix.
6. The plate was incubated for 60 min at room temperature and read with an EnVision®.

Note: Assays can also be miniaturized into 1536-well format.

Materials and Instrumentation

The following tables provide the references of compounds and reagents used or recommended for the characterization of the Human Cannabinoid CB_1 Frozen cells, as well as some advice on how to use these compounds:

Table 1. References of compounds used for functional characterization.

Name	Provider	Cat no	Working Stock Solution
(R)-(+)-WIN 55,212-2	Sigma	W102	10 mM in DMSO
Arachidonoyl ethanolamide	Cayman	90050	144 mM in ethanol
AM251	Tocris	1117	25 mM in DMSO
SR141716A (Rimonabant)	Cayman	9000484	43 mM in DMSO
Forskolin	Calbiochem	344270	50 mM in DMSO

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

Note: The table below lists generic media and additives typically used for Revvity Frozen cells. For product specific media and additives, please refer to the "Recommended Thawing Conditions and Handling of Frozen Cells" section.

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
Calcium and magnesium-free PBS	GIBCO	11010
Standard HBSS (with $CaCl_2$ and $MgCl_2$)	GIBCO	14025
HEPES	MP Biomedicals, LLC	101926
BSA, Protease-free	Sigma	A-3059
Trypsin-EDTA	Hyclone	SH30236.02
Sodium Pyruvate	GIBCO	11360
L-Glutamine	GIBCO	25030
NEAA (non-essential amino acids)	GIBCO	11140
IBMX	Sigma	I-5879
Forskolin	Sigma	F6886

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