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Assay Ready TRP-Channel Expressing Cells - a Flexible Tool to Screen for New Drug Candidates

Summary

Looking for new targets in pain and cancer therapy, the transient receptor potential channels (TRP-channels) gained a lot of interest during the last decade. The ligand-gated calcium channels play an important role in the perception of pain and temperature and are often dysregulated in tumor tissues. They have become appealing targets for Drug Discovery. Recombinant cell lines which stably express different TRP-channels have been successfully used for lead identification and compound profiling.

Assay ready cryopreserved aliquots prepared from these cell lines can be used instantly after thawing without prior cultivation. Here, we demonstrate that Assay Ready Cells prepared from TRP-channel expressing cell lines resemble the pharmacology of cells from continuous culture in different end-point assays. The cells were successfully qualified for plate-based fluorescent calcium-flux assays and for recording of activated ion channel currents using automated patch clamping.



Fig. 1: CHO-huTRPA1 Assay Ready Cells 24 hours after seeding from a frozen aliquot.

Preparation of Assay Ready Cells

Recombinant cell lines stably expressing TRPA1, TRPV1, TRPV3, TRPV4, and TRPM8 had been kindly provided by Assay.Works GmbH (Regensburg).

The cells were expanded in T-Flasks and scaled-up to 10-layer CellSTACKs (Corning). For the final harvest the cells were carefully detached by Accutase (Innovative Cell Technologies) and resuspended in cryopreservation medium containing 5% DMSO. By using a XSD-Biofill (Brooks Life Sciences) the cell suspension was automatically dispensed into cryovials at 10 million cells per vial. Cryopreservation was performed in a Cryomed 7452 (Thermo Scientific) controlled rate freezer using an optimized freezing protocol.

Assay Ready Cells thawed for quality control displayed a viability of greater than 95% and re-entered proliferation without a significant lag phase. 24 hours after seeding, the morphology of the Assay Ready Cells was comparable to cells from a continuously passaged culture (Fig. 1). No elevated levels of debris or dead cells were observed.

Tab. 1: AC₅₀ Values of Calcium Flux experiments recorded on a FLIPR Tetra[®], shown in figure 2.

AC ₅₀ [M]			
Channel	Compound	ARC	fresh
TRPV1	Capsaicine	3.23E-09	4.63E-09
	BCTC	1.61E-09	3.70E-09
TRPV3	2-APB	5.34E-06	1.61E-05
	Nimodipine	8.38E-06	2.97E-06
TRPV4	GSK1016790A	6.14E-10	1.21E-09
	HC067047	5.72E-08	1.85E-07
TRPA1	AITC	1.01E-06	1.56E-06
	A967079	4.80E-08	4.32E-08
TRPM8	Icilin	1.04E-08	2.86E-08
	AMTB	2.13E-06	1.72E-06



Fig. 2: Dose dependent Calcium flux measured in TRP-channels. Assay Ready Cells (blue) and cells from a continuous culture (light blue) after activation (A1-E1) and subsequent inhibition (A2-E2). A: TRPV1, B: TRPV3, C: TRPV4, D: TRPA1, E: TRPM8

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acCELLerate GmbH 1 Jill Court, Bldg. 16/10 Hillsborough, NJ 08844 - USA To demonstrate that Assay Ready Cells can be functionally used, immediately after thawing, the cells were tested in different applications and were compared with cells from a continuous culture.

Calcium Flux in Assay Ready Cells

For a plate-based fluorescence assay detecting intracellular calcium flux, Assay Ready Cells and cultured cells were seeded into 384-well plates at 10.000 cells per well and incubated at 37°C for 24 hours. Serial dilutions of pharmacological reference compounds were added to the cells and the calcium signal was measured in a FLIPR Tetra® (Molecular Devices). All cell lines were activated by their respective ligand and the response was blocked by selective inhibitors in a dose-depended manner. No significant difference in potency or efficacy was observed between Assay Ready Cells and continuously cultured cells (Fig. 2). The experiments were conducted by Assay.Works.

Cation Currents in Assay Ready Cells

TRP-channel expressing Assay Ready Cells were furthermore tested in automated patch clamp experiments.

CHO-huTRPA1 Assay Ready Cells were activated with increasing concentrations of Carvacrol and subsequently blocked with the specific inhibitor AM0902. Current traces of the activated calcium ion channel were recorded on the SyncroPatch 384i (Nanion Technologies GmbH) in perforated patch



Fig 3: Dose-dependent activation and blockage of TRPA1 measured by automated patch clamping on a SyncroPatch 384i in CHO-huTRPA1 Assay Ready Cells.



Fig. 4: Cation current traces (A1-C1) and time courses of currents (A2-C2) for TRPV1 (A), TRPV3 (B) and TRPV4 (C) recorded on a Patchliner.

mode using 4-hole high resistance chips applying a holding potential of -20mV. A success rate of >90% was achieved on 4 consecutive chips. Carvacrol activated the cells with an EC_{50} of 308μ M (n=355). The response was inhibited by AM0902 with an IC_{50} of 48nM (n=349) (Fig. 3).

TRPV expressing Assay Ready Cells were assessed on a Patchliner (Nanion Technologies GmbH). The recordings were performed in whole-cell mode using single-hole, medium resistance chips, applying a holding potential of -60mV and a voltage ramp from -100 to 100mV over 200ms. TRPV1 was activated with Capsaicin, TRPV3 with 2-APB and TRPV4 with GSK1016790A. TRPV1 was blocked with Capsazepine, TRPV3 with Ruthenium Red and TRPV4 with GSK2193874. All Assay Ready Cells could be specifically activated, blocked, and patched with a success rate between 65% for TRPV3 and 89% for TRPV1 (Fig 4). All patch clamp assays were performed by Nanion Technologies.

Discussion

For the cell based discovery and profiling of new drug candidates, the on-time supply of robust and reliable cells is of the essence. Classically cells from a continuously passaged maintenance culture are used which can become a bottleneck in the process. Assay Ready Cells from recombinant cell lines expressing different TRP-channels have been validated for the use in plate-based Calcium flux assays as well as in automated patch clamp assays. No significant difference in the response to the receptor ligands or specific inhibitors was observed which makes the Assay Ready Cells to a reliable and flexible tool in drug discovery.

Since Assay Ready Cells can be prepared and stored in large homogeneous batches and each batch can be prequalified for the particular application, cell culture dependent variances can be substantially excluded from the assay which makes a screening more precise and less prone to failures.

Related Products

CHO-huTRPA1 Assay Ready Cells (Cat. AW210) CHO-huTRPM8 Assay Ready Cells (Cat. AW220) CHO-huTRPV1 Assay Ready Cells (Cat. AW230) CHO-huTRPV3 Assay Ready Cells (Cat. AW240) CHO-huTRPV4 Assay Ready Cells (Cat. AW250)

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