Receptor-Mediated MAPK and Akt Signaling are Maintained in Gamma-Irradiated FroZen Cells

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Abstract

The use of frozen cells for cell-based screening has become widely accepted within the drug discovery community. Separating cell population from the actual screening campaign not only increases your flexibility but also improves the data consistency as the cellular material can be controlled and validated before running the functional assay. One of the methods used to deliver frozen cells as a consumable to the final user is to gamma-irradiate the cells, so that cells cannot resume growth after thawing. This material is currently available for GPCR-expressing cells: AequoZen® cells, validated for calcium flux assays (AequoScreen® or fluorescence assay) and cAMPZen® cells, validated for the LANCE® cAMP assay. The increasing amount of evidence for biased agonism (collateral efficacy and varying potency according to the signal transduction pathway observed), and the search for more physiologically relevant recording of the activity of drugs in development, results in an increasing demand for assays relating to other signaling pathways activated by GPCRs.

Amongst these GPCR-triggered pathways are two important kinase cascades, including MAP Kinase which leads to ERK activation. The location of this kinase downstream from the activation of many GPCRs makes measuring the phosphorylation ideal for evaluating pathway activation/inhibition in the presence of small molecules. AequoScreen® SureFire® is a homogenous assay format for measuring ERK phosphorylation in cells. In this assay system, activated ERK binds a combination of two antibodies, one of which can only bind when ERK is phosphorylated. Only modified proteins that bind both antibodies are detected, using the Alpha technology (PerkinElmer) containing streptavidin coated Donor beads and Protein A coated Acceptor beads. We show here that commercially available frozen, gamma-irradiated cAMPZen cells can be used together with ERK, MEK and Akt AequoScreen® SureFire® assays to assay GPCR stimulation of the MAP Kinase pathway.

Materials and methods

cAMPZen® γ-irradiated FroZen cells (PerkinElmer) CCR7 Cat no. ES-140-CF, Gal: ES-510-CF, Mip: ES-501-CF) were rapidly thawed and seeded at 40,000 cells/well in 96-well plates, in Ham’s F12 medium containing 10% FBS. After 10 min stimulation with the indicated concentrations of agonists, cells were lysed for 10 min with 50 µl of 1x-lysis buffer, (350 rpm plate shaking) and 4 µl of this cell lysate was used to detect ERK, MEK and Akt phosphorylation as recommended in the instructions of the AequoScreen® SureFire® Assay Kits (PerkinElmer Cat no. T9850, T9851 and T9852). The dose-response curves are representative of 2 to 3 independent experiments. Z’ values were calculated using 8 unstimulated, and 8 stimulated (EC50) values.

Conclusions

FroZen, γ-irradiated cells, are a well established product, that can be ordered as a consumable, and readily used to perform functional assays (AequoZen) or cAMP assays (cAMPZen). While, for the Gαi-coupled receptors, an increase of cAMP is detected in such an assay, for Gαq-coupled receptors, an increase of the forskolin-stimulated level of cAMP is detected. This negative read-out may not be the preferred setting to develop an assay that is robust and easy to manage.

The data presented here show that these cAMPZen cells can be used as well in AequoScreen® SureFire® assays, where the stimulation of Gαi-coupled receptors leads to a positive read-out. So γ-irradiation does not prevent the use of such cells for assaying the MEK-ERK or the PI3Kinase pathways.

This will provide additional flexibility for the characterization in multiple assays of drugs in development and allow an easier introduction of the detection of protein agonism (collateral efficacy) in drug discovery programs.

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