AlphaScreen™ PI 3-kinase Assay: A Homogeneous, High-Throughput Assay for Screening Modulators of PI 3-Kinase Activity.

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Abstract

Phosphoinositide 3-kinases (PI 3-kinases) are lipid kinases that have been shown to transduce key signals required for cell proliferation, differentiation and contraction. To simplify and to increase the throughput of screening at PI 3-kinase in drug development, we have combined PerkinElmer’s AlphaScreen™ homogeneous screening technology with Echelon Biosciences specialized phosphoinositide and detection reagents. This simple screening method is based on the binding reaction of a biotinylated PI(3,4,5)P3 probe to a PI(3,4,5)P3 detector-GST fusion protein (Echelon Biosciences peptides). This interaction is detected using the AlphaScreen™ Technology (PerkinElmer Life Sciences #K-1300). This interaction is detected using the AlphaScreen™ Technology (PerkinElmer Life Sciences #K-1300). The interaction of two compounds is detected using the AlphaScreen™ Technology (PerkinElmer Life Sciences #K-1300).

Assays were performed in quadruplicate on 3 separate occasions (unless stated otherwise). Assays were performed on the following standard ribbons in a final volume of 25 µl as follows:

1. Add 2.5 µl of kinase buffer or test compounds*.
2. Add 2.5 µl of enzyme prepared in kinase buffer.
3. Add 10 µl of biotinylated-PIP3 prepared in detection buffer (10nM final).
4. Add 5 µl of PIP3 binding protein prepared in detection buffer (10nM final).
5. Add 5 µl of biotinylated-PIP3 prepared in detection buffer (10nM final).
6. Incubate at 23ºC for 120 minutes in darkness.

Data will be presented showing how the two isoforms of PI 3-kinase (α and γ) can be used to characterize drug hits in a secondary screening laboratory.

1) PI 3-Kinase Alpha

2) Enzyme Titration

3) Substrate Titration

4) Kinase Buffer

5) Standard Curve Assays

6) Standard Curve Assays

7) Enzymatic Assays

8) Enzymatic Assays

9) Enzymatic Assays

10) Pharmacology

11) Precision Assays (Z’ Determination)

12) Conclusion