Rapid and Quantitative Cell-Based Immunoaffinity Kinase Assay in a 96-well Format

A wide range of protein kinases have been implicated in human diseases and, as a result, kinase inhibitors are of great interest as therapeutic drugs. Robust quantitative assays that measure kinase activity in a cellular context are crucial to the mechanistic study of kinase inhibitors. We present an in vitro kinase assay that measures selected kinase activities in cell lysate preparations. In this method immunoprecipitation of a selected kinase and a homogeneous kinase assay (LANCE® method) are carried out in the same well of a 96-well plate. This method offers several advantages over traditional immunoaffinity kinase assays, including a 96-well plate format that enables the handling of multiple samples with consistency and increased throughput, and a homogeneous detection method that avoids the use of multiple wash steps and radioisotopes. We demonstrate the robustness of this technique by evaluating the activity of P38-related tyrosine kinase 3 (p38) in SEM cell lysates. This assay has been extended to other kinases by using target selective antibodies and substrates.

**Assay Principal**

Design of cell-based immunoprecipitation kinase assay in 96-well format. Key assay steps are illustrated above, including LANCE® detection.

**Materials:**
1. 1X Bio Lyso Buffer (CST #9888)
2. 4X Assay Buffer (CST #4900)
3. 10 mM ATP (CST #9804)
4. Genetic Peacer (VGR) (BioDiversity Peptide (CST #4910))
5. Z92 MoAb
6. 1X ECL CST
7. Blocking Buffer
8. 3X-well plate
9. SEM well
10. Sheep polyclonal anti-phosphothreonine rabbit polyclonal Ab (CST #2500)
11. Gastric Precursor (Tyr87) Biotinylated peptide

**Assay Optimization**

Comparison of immunoaffinity kinase assay in two different plate types. Plate B was selected for use in the remainder of the study due to better signal to background ratio (S/B) as compared to plate A.

**Diagram of Assay Principle**

Add cell lysate to capture antibody-coated well and incubate

Wash to remove unbound proteins

Add substrate and incubate

LANCE® detection

**Diagram of Assay Optimization**

Comparison of different plate types shows that plate B has a higher signal to background ratio.

**Summary**

A cell-based immunoprecipitation kinase assay has been developed in a 96-well plate format. Using Flt3 containing SEM cells as an example, we demonstrate that this assay format can selectively measure kinase activity in cell lysate using a homogeneous LANCE® detection method. IC50 values determined using this assay are consistent to those reported in the literature. Compared to traditional immunoprecipitation/kinase assays, this method has the following advantages:

1. A 96-well plate format that eases handling of multiple samples
2. An in-plate immunoprecipitation procedure that replaces a traditional immunoprecipitation method
3. An antibody-based detection method that eliminates radioactive waste generation
4. A homogeneous detection method (LANCE®) that eliminates multiple wash steps