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DDA Platform - Drug Discovery Accelerator

Kinase Screening and Profiling

Boost your kinase research with the most comprehensive set of assay techniques

INTRODUCTION

Kinases are arguably one of the most important drug targets due to the roles their dysregulation, mutations, and overexpression play in all major pathologies, including metabolic, cardiovascular, degenerative, inflammatory, and infectious diseases. Finding the best method for kinase screening and profiling can be a challenging task and will depend on your research objective, the type of kinase, availability of an antibody, the size of the kinase substrate and the analytical instruments available. Creative Bioarray provides a comprehensive set of assay technologies to solve all of your research objectives - from target discovery to compound screening, profiling and hit confirmation. Our breadth of expertise and experience in kinase research enables our scientists to support you every step of the way through the drug discovery process.

KINASE ASSAYS

There are a large number of approaches to evaluate kinase function and activity. In recent years, several different types of kinase assay technology have been developed, each of which have their relative merits and drawbacks.

RADIOMETRIC ASSAY

Radiometric kinase assay using ^{32}P-labelled ATP or ^{33}P-labelled ATP is one of the oldest techniques used to study protein phosphorylation. They rely on the transfer of radioactive phosphate groups from ATP to the substrate by a kinase, allowing detection of activity. The radiolabeled phosphate incorporated into the substrate is directly proportional to the kinase activity.

Radiometric assay formats are the gold standard for kinase screening, which can produce reproducible, high-quality data and directly measure enzyme activity. But they are labor intensive and require safe disposal of radioactive materials, so they are usually not suitable for high-throughput screening.

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Fluorescence Resonance Energy Transfer (FRET) Assay

Fluorescence resonance energy transfer (FRET) involves the transfer of non-radiative energy from a donor fluorophore to a close-proximity acceptor fluorophore. When the donor and acceptor are spatially close to each other, the acceptor molecule will quench any fluorescence emission from the donor molecule. The first step of the FRET kinase assay is to incubate the FRET labeled substrate with ATP and kinase, resulting in the transfer of γ-phosphate to the substrate peptide. In the second reaction step, a site-specific protease is added to the mixture. If the protease cleaves a non-phosphorylated peptide, the fluorescent donor and acceptor will be sufficiently separated to disrupt the FRET, thus generating a fluorescent signal that can be measured as a ratio of donor emission to acceptor emission.

The advantages of FRET are its homogenous format and simple application to HTS. However, the peptide substrate design may be difficult to optimize as the donor and acceptor fluoro-phores need to be within a well-defined distance to transfer energy.

TIME RESOLVED FLUORESCENCE (TRF)

Time-resolved fluorescence (TRF) uses fluorophores with a long decay time, and monitors the fluorescence as a function of time after excitation by a flash of light. Lanthanide ions, such as europium, samarium, and terbium, are often used in this technology due to their longer emission lifetimes (hundreds of microseconds compared to several nanoseconds for conventional organic fluorophores).

The advantage of this technology is that contaminating fluorescent molecules which contribute to background noise have a shorter decay time than lanthanide ions, so the signal is much cleaner. There are several commercially available TRF assays, mainly based either on ELISA assay or combined with FRET assay.

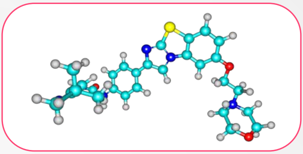
FLUORESCENCE POLARIZATION (FP) ASSAY

Fluorescence polarization (FP), a technique that monitors molecular movement and rotation, is a widely used detection method for kinase inhibitors in HTS. The principle of this assay is that smaller molecules rotate faster than larger molecules. A fluorescently labeled peptide substrate in the solution rotates quickly and so will have a low fluorescence polarization. When the phosphorylation reaction is initiated by addition of a kinase and ATP, any phosphorylated peptide substrates will be recognized by the phospho-antibody. The binding of the antibody will slow down the rotation of the molecule (high fluorescence polarization), and this change in speed can be measured.

Since this is a one-step assay without additional washing steps, it is very suitable for screening a large number of kinase inhibitor candidates. However, one of the major drawbacks for this and other fluorescence-based assays, is that unused fluorescent compounds and labeled substrates may produce high background signals and false positives.

LUMINESCENCE DETECTION

Luminescence-based assay uses firefly protein luciferase to measure the amount of ATP. In the presence of ATP, luciferase converts the substrate luciferin into oxyluciferin, which releases yellow-green photon of light with a spectral maximum of 560 nm. Although this format shows low sensitivity at low ATP concentrations, this effect can be counteracted by using a two-step detection method including 1) stopping the kinase reaction and depleting the remaining ATP, and 2) adding reagents to convert ADP to ATP, which is measured using the coupled luciferase reaction. Luminescence detection methods can accommodate fluorescent compounds, but the inhibitory effect of the compounds on luciferase must be considered.



MOBILITY SHIFT ASSAY

The mobility shift assay takes advantage of the fact that a phosphorylated peptide substrate is more negatively charged than the same substrate in the unphosphorylated state. Therefore, when a mixture of these peptides is subjected to gel electrophoresis, the phosphorylated and unphosphorylated substrates will have different mobility.

Since the difference in charge between the peptide and the substrate is key, the result of mobility shift assay is greatly affected by the size and sequence of the substrate. Therefore, this assay is not suitable for the analysis of larger peptides or whole protein, and works best when the peptide substrate contains only specific phosphorylation site sequences.

CELL-BASED ELISA ASSAY

Cell-based functional kinase screening provides a reliable, quantitative mean for determining the functional inhibitory effects of drug candidates on kinase targets of interest in a physiologically relevant cellular environment. This allows for a more predictive characterization of the organism's physiological response to the drug, and greatly reduces the chances of false positive/negative data.

Enzyme-linked immunosorbent assay (ELISA) can be used to quantitate kinase levels and assess their activity. In this format, the substrate is captured by the membrane and detected by a specific anti-phosphorylated substrate antibody. The detection can also be very sensitive when using antibodies labeled with highly fluorescent dyes.

ELISA is popular because it is generally fast, inexpensive, easy to accommodate multiple samples, and does not require highly specialized equipment. However, the requirement of separation with multiple washing steps limits the use of ELISA in HTS. In addition, the development of high-quality phospho-specific antibodies presents another challenge.

CONCLUSION

A large number of commercially available kinase assays have been developed in recent years based on the technologies described above. Each technology has its strengths and weaknesses, and the most appropriate assay will depend on the aim of the project. For high-throughput screening drug discovery projects, methods that can be easily automated and miniaturized, such as the fluorescence-based assays, are the most suitable. For projects that require a more in-depth profile of the kinase function, a more robust assay such as radiometric assay will provide more reliable results.

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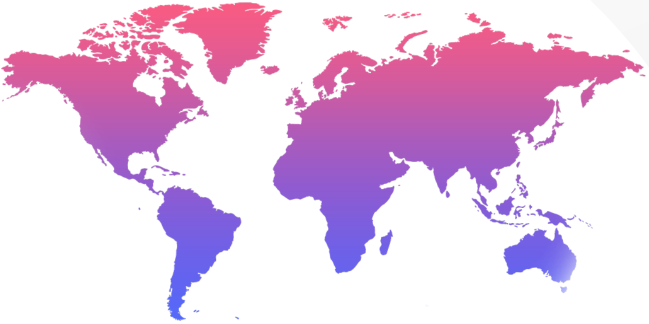

USA

COMPANY: Creative Bioarray
PHONE: 1-631-626-9181
FAX: 1-631-614-7828
ADDRESS: 45-1 Ramsey Road, Shirley, NY 11967, USA
EMAIL: info@creative-bioarray.org

EUROPE

COMPANY: Creative Bioarray
PHONE: 44-208-144-6005
EMAIL: info@creative-bioarray.org