SPA ENZYME ASSAY DESIGN

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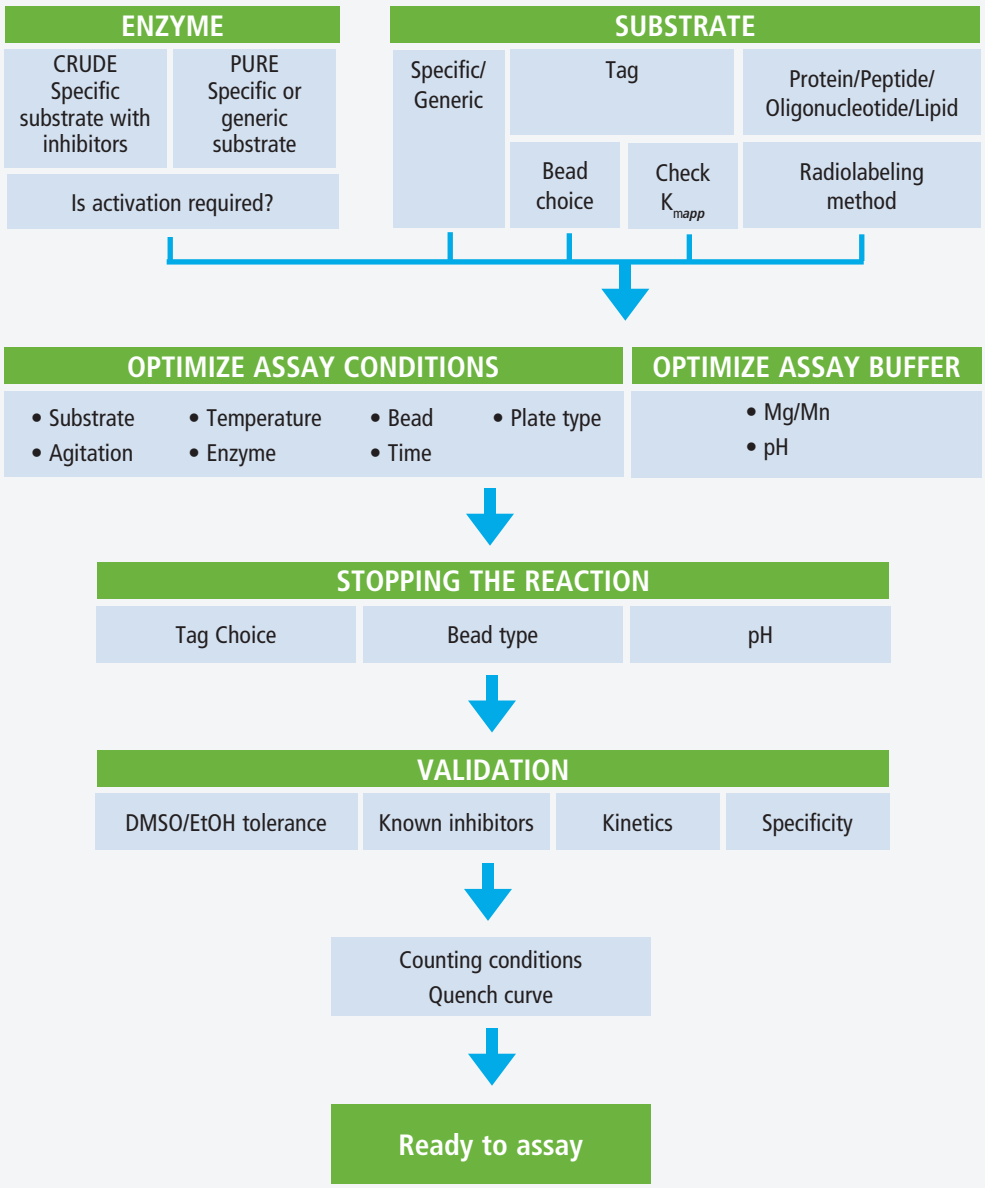

ENZYME

The source of enzyme for activity measurements in SPA assays can be obtained from either cell lysates (crude protein) or from purified preparations (e.g., recombinant protein). In the case of crude enzyme, competing activities may also be present in the cell lysate that could affect the assay performance. The use of specific substrates may overcome this issue, or it may be necessary to add inhibitors to block the competing activities. In the case of pure enzymes, substrate can be either specific or generic. Another factor to consider when setting up the assay is that many enzymes require activation, either by post translational modification or the presence of co-factors, likely present in the cell lysate but for pure preparations it may be necessary to add any known co-factors.

A wide range of molecules have been used as substrates for enzyme SPA, including peptides, proteins, nucleic acids, and lipids. Regardless of the substrate type, it is essential in this assay format that the substrate be captured onto the bead. Biotin is the most commonly used substrate tag that can be captured using Streptavidin-coated beads. Alternatively, the assays can be optimized using substrates containing either His-tags or GST fusion molecules, for capture using Copper chelate beads (RPNQ0095 or RPNQ0096) or Glutathione coated beads (RPNQ0030 or RPNQ0034) respectively. It is also possible to use a specific antibody to capture the substrate or the enzymatically processed assay product. Finally, it is also possible to set up the SPA assay using a non-tag method, such as the ionic capture in the PDE assay (TRKQ7090, TRKQ7100).

During optimization of the SPA assay all tagged substrates should be checked for the effect of the tag on enzyme recognition and action. The K_m of the substrate may be altered by the addition of a specific tag.

For certain enzyme activities, such as proteases and nucleases, radiolabeling of the substrate is also required. This may require the introduction of certain precursor amino acids into peptides. PerkinElmer offers a custom radiolabeling service.

Contact your local sales representative for information related to custom radiolabeling of protein substrates.

OPTIMIZE ASSAY CONDITIONS

The concentration of the various assay components needs to be optimized. In many instances, reaction conditions can be transferred directly from existing assay formats. It should be noted that some assay components will have a direct effect on each other. For example, increasing the mass of substrate will affect the mass of bead required to capture that substrate.

As with any SPA assay, it may be possible to perform the assay in an "on-bead" format (i.e. the substrate is pre-coupled to the bead prior to enzyme incubation) rather than in solution ("off-bead"). Order of addition of reagents may make a difference and should be checked.

OPTIMIZE ASSAY BUFFER

pH and the requirement for co-factors should be assessed.

STOPPING THE REACTION

Stop reagents are required due to the homogeneous nature of SPA. The most commonly used reagents are EDTA, where enzyme activity is dependent of the presence of a divalent metal cation, or a pH shift. It should be noted that the choice of bead coating may influence the composition of the stop reagent. For example, EDTA is not appropriate for use with copper chelate beads (RPNQ0095 or RPNQ0096). It is usually possible to mix the stop reagent and the beads to allow for a single reagent addition.

VALIDATION

The assay should be assessed for tolerance against solvents used for compound addition and against other potentially interfering substances.

Known inhibitors can be used to compare the IC_{50} values obtained in the SPA assay with those published in the literature. The reaction specificity of the assay should be checked by using an alternative methodology, such as HPLC. Kinetic analysis can also be used for assay validation.

COUNTING CONDITIONS

For accurate counting, ensure that the counter settings are correct for both core bead type (PVT or YSi) and the radiolabeled isotope. If assay samples contain color, it is advisable to set up a quench correction curve prior to screening. For large numbers of plates, assay drift may be observed due to the settling of YSi beads. Identical packing conditions should be used for all assay plates.

Please visit **www.perkinelmer.com/SPA** for additional information.

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