SPA MOLECULAR INTERACTION ASSAY DESIGN

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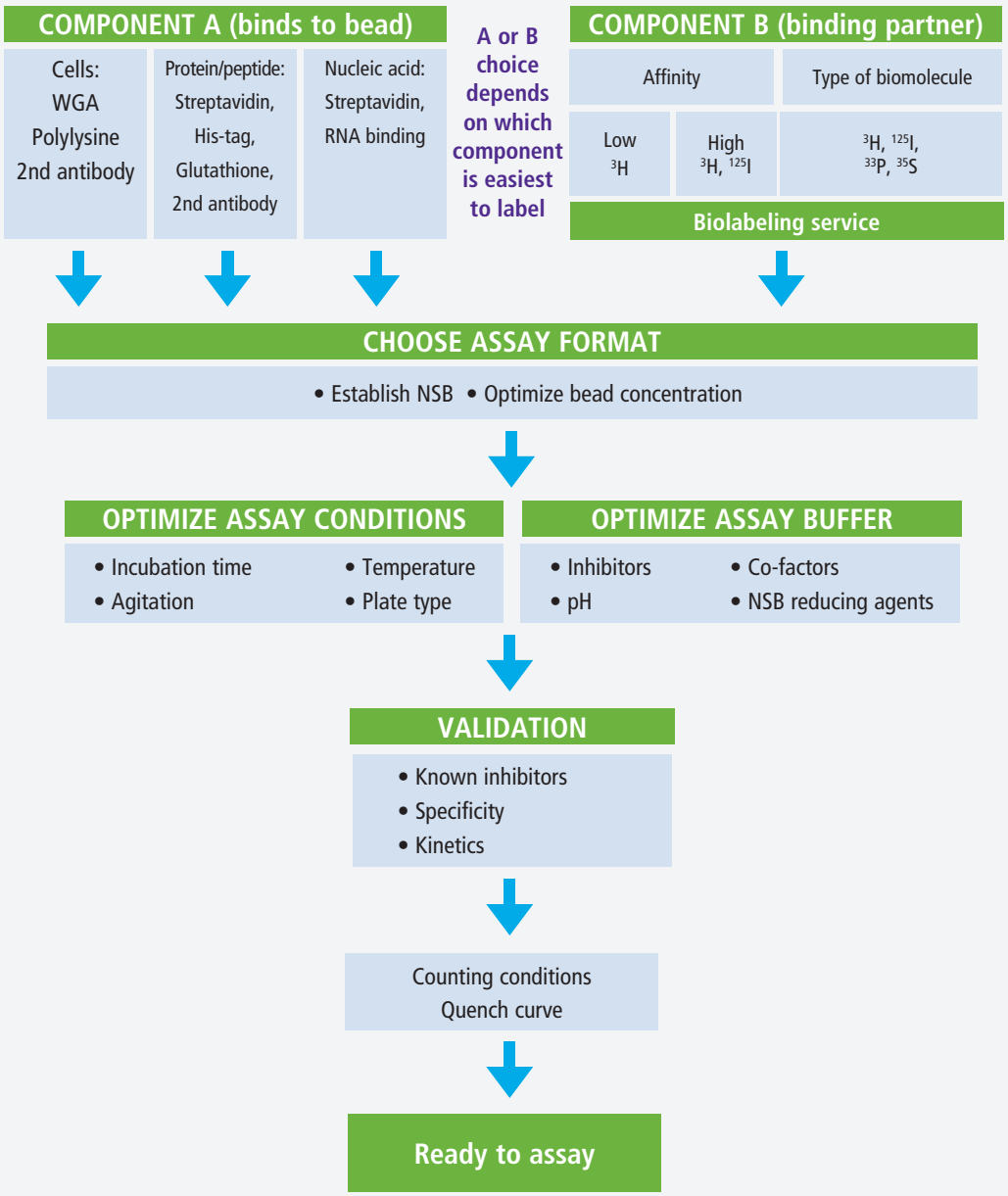
For the Better



BEAD SELECTION

Bead selection depends upon the source of component A which binds to the bead in the molecular interactions assay format.

CELL MEMBRANE BOUND MOLECULE

WGA SPA beads (RPNQ0001, RPNQ0003, RPNQ0004 or RPNQ0011) bind cell membranes via glycoproteins and glycolipids that are components found in cell membranes. For example, 1mg of beads will bind 10–30 µg of membrane protein.

Polylysine SPA beads (RPNQ0010) can be used to bind negatively charged membranes. 1 mg of beads will bind up to 10 µg of membrane protein.

Antibody-capture SPA beads (RPNQ0016, RPNQ0017, RPNQ0018 or RPNQ0019) can be used in conjunction with a specific antibody to capture proteins, peptides, and nucleic acids. They can also be used to capture Ig fusion proteins.

PURIFIED PROTEIN/PEPTIDE

Streptavidin-coated beads can be used to capture biotinylated molecules. 1 mg of SPA bead (RPNQ0007) will capture at least 100 pmol of biotin-labeled molecules.

His-tagged proteins/peptides can be captured onto copper chelate SPA beads (RPNQ0095 or RPNQ0096) and GST fusion proteins/peptides can be captured onto glutathione SPA beads (RPNQ0030 or RPNQ0034).

The binding capacity of the specific bead used will be dependent upon the protein/peptide and should be determined experimentally for each assay.

OLIGONUCLEOTIDES

Oligonucleotides can be bound to SPA beads via biotinylation and capture onto Streptavidin beads (RPNQ0007 or RPNQ0012) or directly bound to the yttrium silicate RNA-binding beads (RPNQ0013). The binding capacity of the beads will be dependent upon the specific oligonucleotide and should be determined experimentally for each assay.

ISOTOPE CHOICE

The type of biomolecule used in the SPA assay can influence the choice of isotope and the method of labeling. For the labeling of oligonucleotides the choice is between ^{3}H or ^{33}P. For labeling of proteins and peptides the preferred choice of isotope is either ^{125}I or ^{3}H.

The affinity of binding between component A and component B is also important in the determination of best isotope to label with. When the affinity is between 10 nM – 10 µM it is best to use a ^{3}H label. When the affinity is less than 10 nM than either ^{3}H or ^{125}I can be used to label the appropriate component.

ESTABLISH ASSAY FORMAT

Establish non-specific binding (NSB) to the bead by incubating the bead with radiolabeled component B and buffer.

Optimize component A:SPA bead ratios to ensure complete capture of all component A present in the reaction. Set up a matrix of bead and component A to identify the combination that produces the optimum signal:background.

There are three possible formats for the assay that can be selected, pre-coupled bead addition, T_0 addition, or delayed addition. Pre-coupling affords the measure of "on" and "off" rates for the molecular interaction. The T_0 addition format involves sequential addition of test samples, radiolabeled component, beads as separate additions. The coupling of one component to the bead occurs simultaneously with the binding of the two components to each other. For the delayed addition format test samples and the radiolabeled component is allowed to equilibrate prior to the addition of the capture beads.

OPTIMIZE ASSAY CONDITIONS

Incubation time must be optimized as SPA binding assays need to fully equilibrate to achieve stable counting conditions. To make this determination it is important to perform a time-course experiment.

Examine the effect of shaking/agitation and temperature on signal and equilibration time. When using yttrium silicate beads, assays will need to be shaken to avoid bead settling.

OPTIMIZE ASSAY BUFFER

Examine the effect of pH and co-factors such as divalent cations, on the generation of signal.

Examine the effect of NSB reducing agents such as BSA, PEI, salts, and detergents on background.

If a loss of signal is observed with time, evaluate the requirement for protease inhibitors to be added to the reaction.

VALIDATION

Establish the specificity (e.g. by the use of negative control cell line). Perform inhibition experiments with known inhibitors to establish IC_{50} values. Perform saturation-binding studies to establish K_D and B_{max} values.

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

PVT SPA beads			
WGA	RPNQ0001	WGA PEI Type A	RPNQ0003
WGA PEI Type B	RPNQ0004	Streptavidin	RPNQ0007
Copper chelate	RPNQ0095	Glutathione	RPNQ0030
Anti-rabbit	RPNQ0016	Anti-mouse	RPNQ0017
Anti-sheep	RPNQ0018	Protein A	RPNQ0019

KITS	
Select-a-Bead Kit	RPNQ0250

Yttrium silicate SPA beads			
WGA	RPNQ0011	Poly-L-Lysine	RPNQ0010
Streptavidin	RPNQ0012	RNA binding	RPNQ0013
Copper chelate	RPNQ0096	Glutathione	RPNQ0034
Arginine binding	RPNQ0101	Anti-rabbit	RPN140
Anti-mouse	RPN141	Anti-sheep	RPN142
Protein A	RPN143		

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