SPA RECEPTOR BINDING STUDY DESIGN

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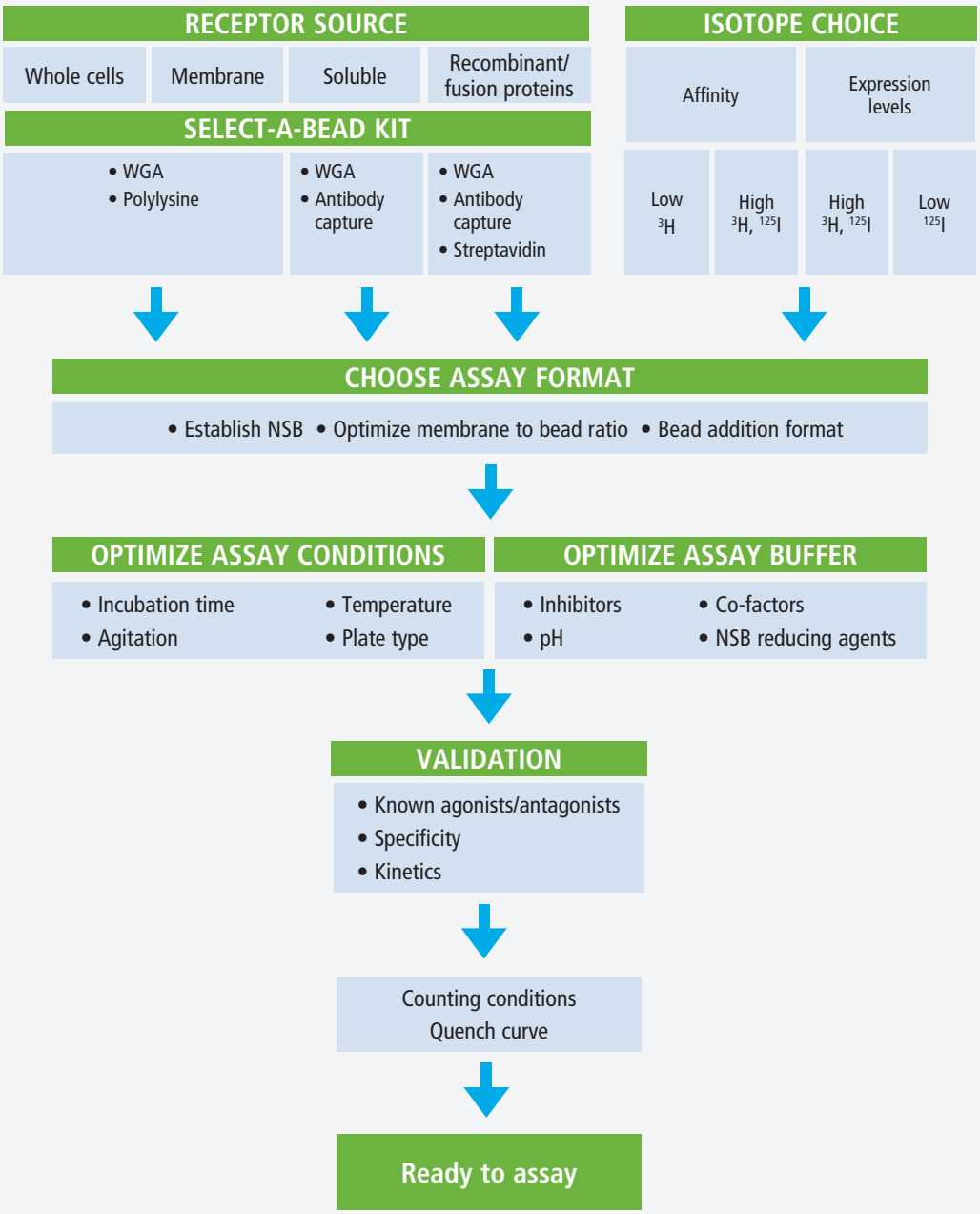

RECEPTOR SOURCE

Ideally use cell membranes or soluble recombinant receptors but whole cells can be used as a receptor source. For membrane preparations, PerkinElmer offers the Membrane Target Systems® which are quality assured frozen membranes from cells that express recombinant or endogenous receptors exclusively from PerkinElmer.

Go to: **www.perkinelmer.com/gpcrmembranes** for a full list of membranes and cell lines.

SELECT-A-BEAD KIT (RPNQ0250)

WGA SPA beads (RPNQ0001, RPNQ0011, RPNQ0003, RPNQ0004) bind glycoproteins and glycolipids in cell membranes. 1 mg of beads will bind 0–30 µg membrane protein.

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

Antibody capture SPA beads (Protein A-coated beads RPNQ0019 or RPN143) can be used in conjunction with a specific antibody to capture soluble and solubilized receptors. They can also be used to capture Ig-fusion proteins.

Streptavidin-coated SPA beads (RPNQ007 or RPNQ0012) can be used to capture biotinylated receptor protein. 1 mg of beads will capture at least 100 pmol of biotin.

ISOTOPE CHOICE

Affinity of ligand binding determines the radiolabel choice.

For studies where the receptor density is high, either ^{3}H or ^{125}I-labeled radioligands may be used if the affinity for the receptor is also high (< 10 mM). But, if the affinity of the ligand for the receptor is low (in the range of 10 nM – 10 µM), ^{125}I is probably the radioisotope of choice for labeling.

RECEPTOR EXPRESSION LEVELS

If using ^{3}H-labeled ligands with low specific activity (20 – 80 Ci/mmol) then higher expression levels are required (500,000 receptors per cell) corresponding to densities greater than 2 pmoles receptor protein/mg of membrane protein.

If using ^{125}I-labled ligands with high specific activity (~ 2,000 Ci/mmol) then lower expression levels can be tolerated (50,000 receptors per cell) corresponding to densities of approximately 200 fmol receptor protein/mg of membrane protein.

ESTABLISH ASSAY FORMAT

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 receptor binding. The T_0 addition format involves sequential addition of test samples, radio-ligand, membrane and bead as separate additions. The coupling of membrane to beads occurs simultaneously with the ligand-binding. This is the most widely used format for screening assys that are automated. For the delayed addition format test samples, radio-ligand and membrane are allowed to equilibrate prior to the addition of beads.

Optimize receptor:SPA bead ratios to ensure complete capture of all the receptor present. Set up a matrix of bead and receptor to identify the combination that produces the optimal signal:background ratio.

OPTIMIZE ASSAY CONDITIONS

Incubation time must be optimized because receptor-binding SPA assays need to be fully equilibrated to achieve stable counting conditions. Perform a time course experiment.

Examine effect of shaking/agitation and temperature on the signal and equilibration time. Receptor-binding SPA assays using yttrium silicate (YSi) beads (RPNQ 0011, RPNQ0010, RPN143, RPNQ0012) will need to be shaken to prevent clumping and poor ligand binding results.

OPTIMIZE ASSAY BUFFER

Examine the effect of NSB reducing agents such as BSA, salts (NaCl or $MgCl_2$), detergents (e.g, Triton-X100) and polyethylenimine coating (RPNQ0003 or RPNQ0004 vs. RPNQ0001) on the level of background signal when added to the reaction buffer.

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

If a loss of signal is observed with time, evaluate the requirement for the addition of protease inhibitors in the reaction.

VALIDATION

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

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

Yttrium silicate SPA beads			
WGA	RPNQ0011	Poly-L-Lysine	RPNQ0010
Anti-rabbit	RPN140	Anti-mouse	RPN141
Anti-sheep	RPN142	Protein A	RPN143
Streptavidin	RPNQ0012		

KITS	
Select-a-Bead Kit	RPNQ0250

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

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