

## GE HEALTHCARE – Advanced Systems

### Cell Factory – Quote Request Form

**Service type: SPA imaging receptor binding assay**

#### Customer details:

a) **Name:** [Click here and start typing text]

b) **Company:** [Click here and start typing text]

c) **Contact information:**

**Telephone:** [Click here and start typing text]

**Cell Phone:** [Click here and start typing text]

**email:** [Click here and start typing text]

**Fax:** [Click here and start typing text]

#### Recommendations and requirements:

1. Fully configured filter binding or SPA assays will be converted to SPA imaging format using the steps shown in the table on page three. Customers should be willing to share all relevant experimental details of their assay with GE Healthcare.
2. Preferred format is either 96 or 384-well.
3. Assays will be counted on the LEADseeker platform.
4. Work will employ available receptor (membrane) source (typically supplied by customer) and a commercially available radioligand.
5. Receptor expression levels in the cell/membrane preparation supplied will be high enough to ensure a workable S:N ratio (typically 5:1 minimum) in either filter or SPA assay formats. Typically, expression levels in the region of 50,000 receptors per cell are required for [<sup>125</sup>I] ligands, whereas for tritium ligands expression levels of the order of 500,000 receptors per cell are required.
6. The Kd and Bmax for the respective radioligand and receptor should be known.



## Assay Target Details:

- a) Name of receptor assay target: [Click here and start typing text]
- b) Do you have a supply of receptor eg cells/membranes/other?  Y  N
- c) Do you source receptor from a commercial source?  Y  N
- d) Would you like GE Healthcare to provide you with a quotation for developing a cell line and preparing cell membranes?  Y  N
- e) Would you prefer to work with membranes from a  stable or  transiently transfected cell line?
- f) Do you have a preferred radioligand for this receptor?  Y  N

If Yes, please specify: [Click here and start typing text]

- g) Would you like GE Healthcare to provide you with a quotation for custom synthesizing this radioligand?  Y  N
- h) Do you have an existing filter and/or SPA assay for this target?  Y  N

If Yes, please provide details separately.

- i) What is your preferred assay format?  
 96  384  1536
- j) Do you have a preferred type of microplate?  Y  N

If Yes, please specify: [Click here and start typing text]

- k) What is your minimum acceptable S:N? [Click here and start typing text]
- l) What is your minimum acceptable Z'? [Click here and start typing text]

- m) Do you have a preferred assay format? e.g. precoupled membrane to SPA imaging bead format?  Y  N

If yes, please provide details:[Click here and start typing text]



	DESCRIPTION	COMMENTS	Y/N
<b>ASSAY FEASIBILITY</b>			
1	Selection of assay buffer and SPA imaging bead type	The assay buffer employed for SPA or filter binding format assays will usually prove suitable for LEADseeker assays. GE Healthcare's range of appropriate polystyrene (PS) and Yttrium Oxide (YOx) beads will be evaluated at this stage.	[Y/N]
2	Optimization of membrane to bead ratio and ligand amount	Once the bead type has been selected the next step is to determine the actual capacity of the imaging bead for receptor membrane; the membrane to bead ratio. A number of matrix experiments of the imaging bead selected in step 1 and the receptor membrane is performed.	[Y/N]
3	Optimization of bead and membrane amount	Once the membrane to bead ratio has been established, the optimum bead and membrane amount should be determined. This is done by premixing the bead and membrane at the ratio established in step 2 and diluting in assay buffer to give a series of dilutions containing varying amounts of bead and membrane at a fixed ratio of membrane to bead.	[Y/N]
4	Optimisation of assay buffer	Reagents such as BSA (0.1-0.5% w/v) or NaCl (10-100mM) may be added to reduce non-specific binding of the ligand to the bead or membrane. Protease inhibitors may be added to the buffer to improve signal stability.	[Y/N]
5	Time course and assay signal stability	Once the assay has been configured in terms of bead and membrane additions it is important to perform a time course analysis to ensure that the assay is read at equilibrium and that the assay signal is stable at equilibrium. This is simply done by setting up the assay with the predetermined amounts of bead and membrane and imaging at intervals for ~24 hours	[Y/N]
	<b>GO/NO GO DECISION POINT</b>	The aim at this stage is to have met the desired (and agreed) assay specifications in terms of S:N, assay signal etc in the format specified at the start of the work.	
<b>ASSAY VALIDATION</b>			
6	Determination of the assay solvent tolerance	DMSO only. Other solvents as requested.	[Y/N]
7	Saturation binding analysis	Saturation binding is performed. The assay is set up with increasing concentrations of radiolabelled ligand. Three independent experiments are performed and Bmax and Kd determined.	[Y/N]
8	Competition binding analysis (pharmacology)	Determination of IC50 for a number of competing ligands specified. Three independent experiments are performed.	[Y/N]
9	Z' Analysis	To confirm the robustness of the assay a Z' analysis is performed. The assay is set up with a number of replicate values each for "total" and NSB wells. Between 50 and 100 replicates wells are typically set up to determine Z'.	[Y/N]



10	Determination of association and dissociation kinetics.	On and off rate analysis is performed.	[Y/N]
11	Produce Full Report/Protocol	Includes all methods and data analysis.	[Y/N]
<b>ADDITIONAL REQUIREMENTS</b>			
A			
B			

