Techniques for Studying Receptor – Ligand Interactions

Service	Description	Recommended for	Sample / Materials	Deliverables	Pros	Cons
Radioligand Binding	Radiometric technique used to measure ligand-receptor binding parameters (96-well plates)	➢ Determining ligand-receptor affinity and receptor expression levels (saturation assays) ➢ Determining affinity of unlabelled test compounds competing with a radiolabelled compound (competition assays) ➢ Determining kinetics for association and dissociation of radiolabelled ligand to the receptor ➢ Determining protein and antibody affinity to membrane targets ➢ Evaluating allosteric binding	Receptor sources: ➢ Cell membranes (from wild-type or transfected cells) ➢ Tissue homogenates ➢ Whole cells ➢ Immobilized recombinant receptors Radioligands: ➢ [^{3}H], [^{35}S], [^{125}I]-labelled compounds (commercially available or custom synthesized)	➢ K_d ➢ B_{max} ➢ IC_{50} and K_i ➢ k_{on} ➢ k_{off}	➢ High-sensitivity ➢ High-throughput ➢ Robust ➢ Works well on membrane receptors ➢ Tritium labelling can be used for small molecules without changing structure ➢ ^{125}I custom labelling can be used for peptides, proteins, antibodies	➢ Works best when radioligand K_d<~50 nM ➢ A suitable high-affinity radioligand may need to be custom synthesized (if not commercially available) ➢ Cytoplasmic (soluble) proteins need to be first immobilized
Receptor Autoradiography	Radiometric technique based on quantitative phosphor imaging of radioactivity in tissue sections from untreated or drug-treated animals (glass slides)	➢ Determining distribution of receptor-ligand binding sites ➢ Quantifying regional receptor levels ➢ Conducting *ex vivo* receptor occupancy studies to determine the percentage of receptor occupancy produced by a given dose of drug administered to the live animal	Receptor sources: ➢ Rodent tissue or organs from untreated or drug-treated animals ➢ Human tissues (post-mortem or from surgical removal) ➢ NHP tissues (post-mortem) Radioligands: ➢ [^{3}H], [^{35}S], [^{125}I]-labelled compounds (commercially available or custom synthesized)	➢ K_d ➢ B_{max} ➢ % Receptor occupancy versus dose ➢ ED_{50}	➢ High-sensitivity ➢ Low amount of tissue required ➢ Can be used to detect binding to small tissue regions ➢ For *ex vivo* occupancy studies, lower cost than PET approaches and applicable to a wider range of receptors ➢ Tritium labelling can be used for small molecules without changing structure ➢ ^{125}I custom labelling can be used for peptides, proteins, antibodies	➢ Receptor should be present at sufficient abundance in the tissues ➢ A suitable high-affinity radioligand may need to be custom synthesized (if not commercially available) ➢ *Ex vivo* studies may underestimate receptor occupancy if test (unlabelled) drug dissociates appreciably from the receptor during the *in vitro* incubation period in radioligand
Surface Plasmon Resonance (SPR)	Label-free optical detection technique used to measure interacting biomolecules kinetics parameters (sensor chip)	➢ Testing antibody-antigen interaction ➢ Determining binding kinetics parameters of a variety of molecules (proteins, carbohydrates, small molecules etc.)	Ligands and analytes: ➢ Compounds ➢ Peptides ➢ Proteins and antibodies ➢ Saccharides Sample media: ➢ Buffer ± solvent ➢ Serum ➢ Plasma	➢ K_d ➢ k_{on} ➢ k_{off} ➢ Selectivity/cross-reactivity ➢ Binding inhibition ➢ Analyte concentration	➢ High-sensitivity ➢ High-reproducibility ➢ Label-free detection ➢ Low sample required ➢ Tolerance for sample impurity ➢ Real-time monitoring	➢ Not suitable for whole cells ➢ Works best with non-membrane proteins ➢ Binding response is dependent on target and ligand MW ratio ➢ Small molecule binding to large receptor proteins hard to detect
ELISAs	Immunoassay to detect target proteins using antibodies (96-well plates)	➢ Determining target protein expression levels ➢ Confirming cell surface expression of membrane receptors ➢ Assessing effect of drug treatments on the target expression profile	Target protein sources: ➢ Tissue homogenates ➢ Cell lysates ➢ Plasma	➢ Target concentration ➢ Relative comparison of expression levels between tissues/treatments	➢ High-sensitivity ➢ High-specificity ➢ Label-free detection	➢ Lengthy protocols reduce high-throughput capabilities ➢ Relies on availability of antibodies that bind the target proteins
Fluorescence Assays	Fluorescence-based assays used to measure downstream signalling of receptors upon ligand binding in live cells (96-well/384-well plates)	➢ Determining the effect of ligand-receptor interaction on cell signalling pathway(s) ➢ Profiling the pharmacology of the compound/receptors (agonist, inverse agonist, antagonist, allosteric modulators etc.)	➢ Live cells (endogenous or transfected receptors)	➢ E_{max} ➢ EC_{50} ➢ IC_{50}	➢ High-throughput ➢ Suitable for functional studies	➢ Some test molecules may exhibit intrinsic fluorescence (quenching) that might reduce accuracy and sensitivity ➢ Background fluorescence from cells may increase the signal-to-noise ratio
Cellular Uptake & Release	Radiometric technique used to measure transport of molecules into cells or release of labelled molecules from cells (96-well plate for uptake/perfusion chambers for release)	➢ Determining cellular uptake and release of labelled substrates, drugs, neurotransmitters or proteins	➢ Live cells ➢ Synaptosomes Radioligands: ➢ [^{3}H], [^{14}C], [^{35}S], [^{125}I]-labelled compounds (commercially available or custom synthesized)	➢ Compounds potency and efficacy against membrane transporters ➢ Compounds efflux from cells ➢ IC_{50} and K_i for test compounds to inhibit cellular uptake of radiolabelled substrates ➢ V_{max} and K_m for transport of labelled substrates	➢ High-sensitivity ➢ Robust ➢ Uptake and release can be determined over time ➢ Tritium labelling can be used for small molecules without changing structure ➢ ^{125}I custom labelling can be used for peptides, proteins, antibodies in uptake assays	➢ Low throughput relative to radioligand binding assay approaches ➢ A suitable high-affinity radioligand may need to be custom synthesized (if not commercially available) ➢ Subcellular localization of labelled substrate cannot be determined without further processing