RECEPTOR-LIGAND INTERACTIONS

Which technique is right for your study?

	Description	Deliverables	Sample/ Materials	PROs and CONs
Radioligand Binding	Radiometric technique used to measure ligand receptor binding parameters (96-well plates)	• K_d • B_{max} • IC_{50} • K_i • k_{on} and k_{off}	Receptor sources Radioligands	Pros Cons
Receptor Autoradiography	Radiometric technique based on quantitative phosphor imaging of radioactivity in tissue sections from untreated or drug-treated animals (glass slides)	• K_d • B_{max} • %Receptor occupancy versus dose • ED_{50}	Receptor sources Radioligands	Pros Cons
Surface Plasmon Resonance (SPR)	Label-free optical detection technique used to measure interacting biomolecules kinetics parameters (sensor chip)	• K_d • k_{on} • k_{off} • Selectivity/crossreactivity • Binding inhibition • Analyte concentration	Ligands and analytes Sample media	Pros Cons
ELISAs	Immunoassay to detect target proteins using antibodies (96-well plates)	• Target concentration • Relative comparison of expression levels between tissues or treatments	Target protein sources	Pros Cons
Fluorescence Assays	Fluorescence-based assays used to measure downstream signalling of receptors upon ligand binding in live cells (96-well/384-well plates)	• E_{max} • EC_{50} • IC_{50}	Live cells (endogenous or transfected receptors)	Pros Cons
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)	• 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	• Live cells • Synaptosomes Radioligands	Pros Cons

Radiolidand binding assay are recommended for:

- 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



RADIOLIGAND BINDING

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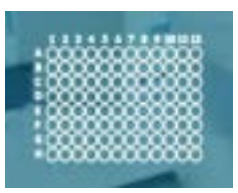
Suitable receptors for radioligand binding can be obtained from:

- Cell membranes (from wild-type or transfected cells)
- Tissue homogenates
- Whole cells
- Immobilized recombinant receptors



RADIOLIGAND BINDING

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Suitable radioligands for radioligand binding are:

- [^{3}H], [^{35}S], [^{125}I]-labelled compounds (commercially available or custom synthesized)



RADIOLIGAND BINDING

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Radioligand binding advantages:

- High-sensitivity
- High-throughput
- Robust
- Works well on membrane receptors
- ^{3}H labelling can be used for small molecules without changing structure
- ^{125}I custom labelling can be used for peptides, proteins and antibodies

RADIOLIGAND BINDING

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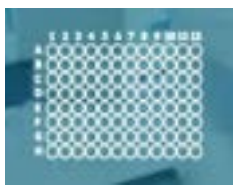
Radioligand binding disadvantages:

- 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



RADIOLIGAND BINDING

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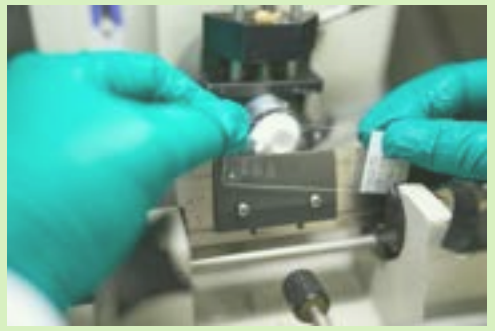
Receptor autoradiography is recommended for:

- 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
AUTORADIOGRAPHY

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Suitable receptors for receptor autoradiography can be obtained from:

- Rodent tissue or organs from untreated or drug treated animals
- Human tissues (postmortem or from surgical removal)
- NHP tissues (postmortem)

RECEPTOR
AUTORADIOGRAPHY

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Suitable radioligands for receptor autoradiography are:

- [^{3}H], [^{35}S], [^{125}I]-labelled compounds (commercially available or custom synthesized)

RECEPTOR AUTORADIOGRAPHY

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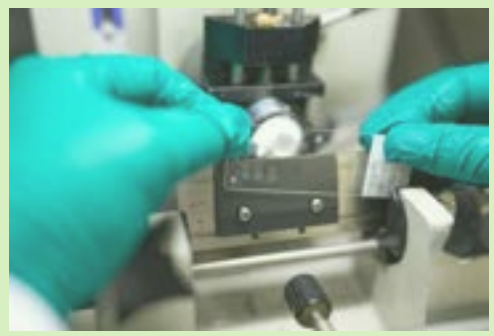
Receptor autoradiography advantages:

- 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
- ^{3}H labelling can be used for small molecules without changing structure
- ^{125}I custom labelling can be used for peptides, proteins and antibodies





RECEPTOR AUTORADIOGRAPHY

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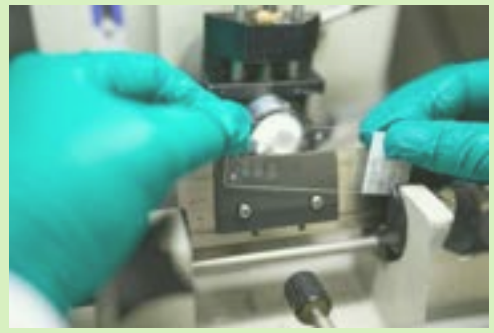
Receptor autoradiography disadvantages:

- 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





RECEPTOR AUTORADIOGRAPHY

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Surface Plasmon Resonance (SPR) is recommended for:

- Testing antibody-antigen interaction
- Determining binding kinetics parameters of a variety of molecules (proteins, carbohydrates, small molecules etc.)

SPR
(BIACORE)

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Suitable ligands and analytes for SPR are:

- Compounds
- Peptides
- Proteins and antibodies
- Saccharides

SPR
(BIACORE)

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Suitable sample media for SPR:

- Buffer ± solvent
- Serum
- Plasma

SPR
(BIACORE)

SPR advantages:

- High-sensitivity
- High-reproducibility
- Label-free detection
- Low sample required
- Tolerance for sample impurity
- Real-time monitoring

SPR (BIACORE)

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SPR disadvantages:

- 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

SPR
(BIACORE)

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ELISAs assays are recommended for:

- Determining target protein expression levels
- Confirming cell surface expression of membrane receptors
- Assessing effect of drug treatments on the target expression profile



ELISAs

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Target proteins for ELISAs can be obtained from:

- Tissue homogenates
- Cell lysates
- Plasma



ELISAs

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ELISAs advantages:

- High-sensitivity
- High-specificity
- Label-free detection



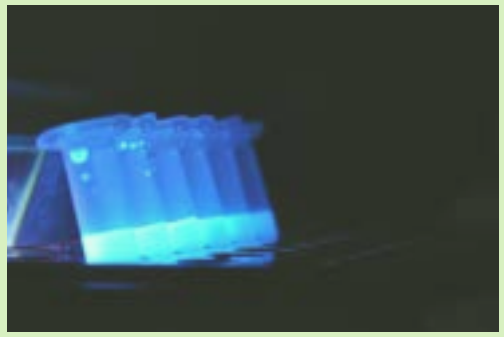
ELISAs

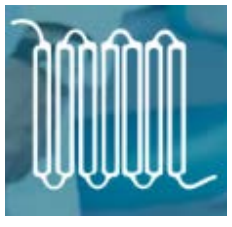
ELISAs disadvantages:

- Lengthy protocols reduce high-throughput capabilities
- Relies on availability of antibodies that bind the target proteins

Fluorescence assays are recommended for:

- 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.)

FLUORESCENCE ASSAYS

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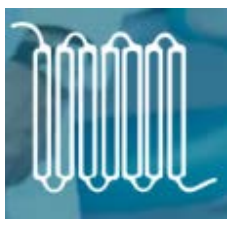
Fluorescence assay advantages:

- High-throughput
- Suitable for functional studies



FLUORESCENCE ASSAYS

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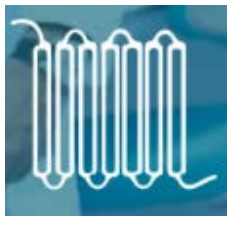
Fluorescence assay disadvantages:

- 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





FLUORESCENCE ASSAYS

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Cellular uptake & release assays are recommended for:

Determining cellular uptake and release of labelled substrates, drugs, neurotransmitters or proteins

CELLULAR UPTAKE & RELEASE

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Suitable radioligands for cellular uptake & release are:

[^{3}H], [^{14}C], [^{35}S], [^{125}I]-labelled compounds (commercially available or custom synthesized)



CELLULAR UPTAKE & RELEASE

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Cellular uptake & release advantages:

- High-sensitivity
- Robust
- Uptake and release can be determined over time
- ^{3}H labelling can be used for small molecules without changing structure
- ^{125}I custom labelling can be used for peptides, proteins, and/or antibodies in uptake assays

CELLULAR UPTAKE & RELEASE

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Cellular uptake & release disadvantages:

- 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

CELLULAR UPTAKE & RELEASE

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