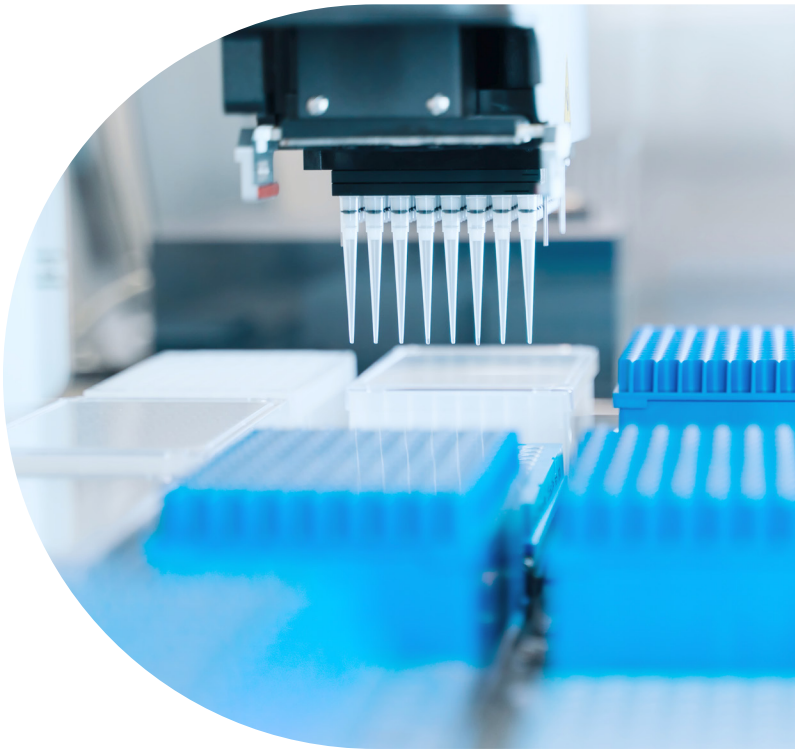
Fact Sheet

Early Hit-to-Lead ADME Screening Bundle

Bundled screening assays to accelerate candidate selection in drug discovery

In vitro ADME screening during the lead optimization stage of drug discovery positively impacts drug candidate selection with an enhanced probability of success in clinical trials. Since most new drug candidates fail during preclinical and clinical development, and the late stage of the drug development cycle can be a lengthy and costly process, any means of identifying drug candidates with optimized ADME and pharmacokinetics properties in the discovery stage will have a significant impact on the drug discovery process overall.



Focused on solutions to address DMPK issues and to enable the success of our clients

Our scientists routinely conduct industry standard *in vitro* metabolism and DDI-based assays, including highly automated ADME *in vitro* screens. We can help drive your discovery phase Structure Activity Relationship (SAR) by optimizing for ADME properties, in parallel to your receptor binding potency and selectivity, for more rapid identification of high-quality drug candidates.

Metabolic stability, risk assessment for inhibiting key Cytochrome P450 enzymes, and cell permeability are three main early hit-to-lead ADME screening assays that all New Chemical Entities (NCEs) are tested for in the industry in effort to optimize key ADME properties.

In vitro ADME screening services: Early hit-to-lead ADME screening bundle

Intrinsic clearance assay in liver microsomes

- Liver microsomes; species selectable
- Typical turnaround time is ≤10 business days
- Test article prepared at 10mM in DMSO
- Substrate and positive control incubated at 0.3µM
- Incubations performed in HLMs at a protein concentration
- Incubation performed using 1mM co-factor (NADPH)
- Incubation time points (+NADPH): 0, 5, 15, 30, 45 minutes
- 45 min negative control (-NADPH) for recovery assessment
- Samples analysis and quantitation by LC/MS-MS
- Deliverables: Unscaled intrinsic clearance, recovery
- Results provided via electronic spreadsheet format

CYP single concentration cocktail assay (CYP 2C9, 206,3A4)

- Human Liver Microsomes (HLM)
- Typical turnaround time is ≤10 business days
- Inhibitors prepared at 10mM in DMSO
- CYP3A4 — Midazolam (5µM), CYP2D6 — Bufuralol (10µM), CYP2C9 — Diclofenac (10µM); substrates incubated as a cocktail
- Test article and positive control inhibitor incubated at 10µM
- Incubations performed in HLMs at a protein concentration of 0.05mg/mL
- Incubation performed using 1mM co-factor (NADPH)
- Incubation time: 3 minutes
- LC-MS/MS analysis using cocktail of heavy labeled internal standard for each metabolite
- A decrease in the formation of the metabolites compared to vehicle control is used to calculate a percent inhibition value
- Samples analysis and quantitation by LC-MS/MS
- Deliverables: Percent inhibition at 10µM
- Results provided via electronic spreadsheet format

MDCK II Bi-directional permeability assay**

- MDCK II cell line (Madin-Darby Canine Kidney cells) — Sigma Aldrich (ECACC)
- Typical turnaround time is <15 business days
- Test article prepared at 10mM in DMSO
- Substrate and positive control incubated at 1µM in duplicate
- Incubations performed in both A:B and B:A in the presence of Cyclosporin A (P-gp inhibitor)
- Incubations carried out in cell culture incubator at 37˚C/5% CO^2/95% RH
- Samples taken from the A:B plate and B:A plate following a 3 hour incubation
- A 9-pt concentration curve is prepared for quantitation of samples (2000, 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.81nM)
- Dextran Texas Red (DTR) used as monolayer integrity check
- Sample analysis and quantitation by LC-MS/MS
- Deliverables: Papp in both A:B and B:A direction, BA:AB ratio, mass balance using both pre and post dose solutions), percent cell leakage (% DTR)
- Results provided via electronic spreadsheet format

**Xiannu Jin, et al. Comparison of MDCK-MDR1 and Caco-2 cell based permeability assays for anti-malarial drug screening and drug investigations, J Pharm Tox Methods, 2014, 70 (2), 188-194.

Protein binding assays

Equilibrium dialysis — Equilibrium dialysis allowing many matrices to be analyzed (plasma, brain, microsomes, tissues)

- HT dialysis or Rapid Equilibrium Dialysis (RED device)
  - » Typical turnaround time is <15 business days
  - » Test article prepared at 10mM in DMSO
  - » Test article and positive control tested at 1µM in triplicate
  - » Incubations carried out in cell culture incubator at 37˚C/5% CO^2/95% RH for 4.5 hours
  - » Sample analysis and quantitation by LC-MS/MS
  - » A 6-pt concentration curve is prepared for quantitation of samples (5000, 2000, 200, 20, 5, 2.5nM)
  - » Deliverables can include fraction unbound/bound and recovery

TRANSIL — Proprietary technique developed by Sovicell — allows for analysis on drug absorption as well as binding, can be used to analyze membrane or plasma proteins

- Typical turnaround time is <15 business days
- Technique suitable for small and large molecules
- Resembles typical physiological protein binding conditions using silica beads expressing specific proteins
- Sample analysis and quantitation be LC-MS/MS
- Deliverables: Fraction unbound/bound, Kd

About IQVIA Laboratories bioanalytical and ADME services

IQVIA Laboratories operates one of the world's largest and most respected bioanalytical and ADME laboratory networks. From our global locations, we serve many of the largest pharmaceutical, specialty pharmaceutical and biotechnology companies in North America, South America, Europe and Asia. Our highly trained scientists utilize a range of leading-edge technology, automation and state-of-the-art techniques.

***In vitro* ADME assays and metabolite identification services** in support of rapid drug discovery ADME property optimization and regulatory filings.

Immunoassay services for the quantitative determination of large molecule therapeutics using ligand binding technologies in support of Pharmacokinetic (PK) studies. Immunogenicity assessments for pre-clinical and clinical studies including tiered based approaches, neutralizing assays and isotyping assays.

Bioanalytical Liquid Chromatography Mass Spectrometry neutralizing assays and isotyping assays (LC-MS) services for the quantitative determination of small molecule, peptide and macromolecule therapeutics in support of Pharmacokinetic (PK) studies.

Fit-for-Purpose biomarker services for the quantitative determination of discovery, pre-clinical and clinical biomarkers using LC-MS, Immunoaffinity-LC-MS and immunoassay technologies in GLP compliant facilities.

Contact us today to learn how we can help you characterize the drug disposition properties of your small and large molecule platforms and to enable successful drug discovery.

IQVIA LABORATORIES

CONTACT US
U.S.: 1-833-793-5298
UK: +44 800 028 9326
Email: BioAinfo@iqvia.com
labs.iqvia.com