

# Plasma Protein Binding Assays

The pharmacokinetic and pharmacodynamic properties of drugs are largely a function of the reversible binding of drugs to plasma or serum proteins. Such proteins include albumin,  $\alpha_1$ -acid glycoprotein, lipoproteins and  $\alpha$ ,  $\beta$ , and  $\gamma$  globulins. Generally, only the unbound drug is available for diffusion or transport across cell membranes, and for interaction with a pharmacological target (e.g. receptor, ion channel, transporter, enzyme). As a result, the extent of plasma protein binding of a drug influences the drug's action as well as its distribution and elimination.

Highly plasma protein bound drugs are confined to the vascular space, thereby having a relatively low volume of distribution. In contrast, drugs that remain largely unbound in plasma are generally available for distribution to other organs and tissues, resulting in large volumes of distribution. The binding of drugs to proteins both in the vascular space and/or the extravascular space results in a decrease in drug clearance and a prolonged drug half-life. Only the unbound drug is available for glomerular filtration and, in some cases, hepatic clearance. However, for high extraction ratio drugs, clearance is relatively independent of protein binding.

NoAb BioDiscoveries offers plasma or serum protein binding assays using the equilibrium dialysis method. Equilibrium dialysis studies are offered using a 96-well Teflon dialysis unit. Combined with LC-MS/MS analysis for test compound quantification, the 96-well format is a rapid throughput method that is excellent for screening compounds to identify those with high, medium and low binding fractions. The system can be used to determine the fraction of a compound bound and unbound to plasma proteins and the effect of concentration on the extent of binding.

## Key Features of the Assay

- can use whole plasma or an individual plasma protein
- plasma from difference species are available, including human, dog, rabbit, rat, and mouse
- using the 96-well format, the assay achieves a higher throughput and can be used to screen small libraries of compounds

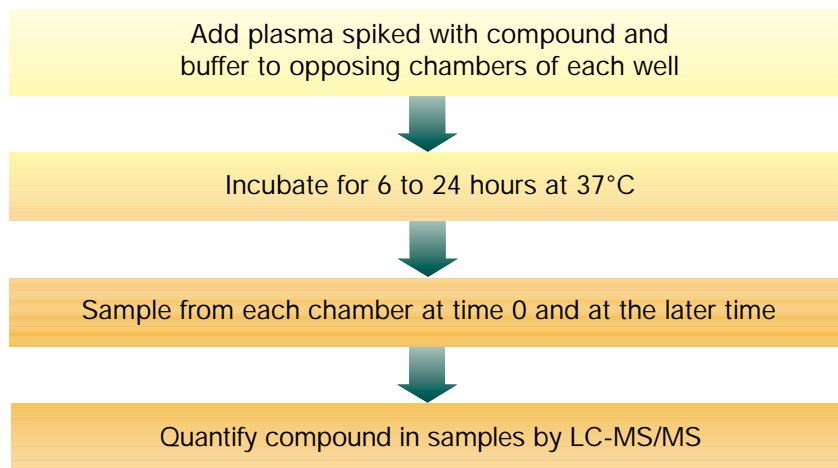
## Assay Applications

- early prioritization of compounds or compound series
- assessment of plasma proteins which contribute to the binding of the compound
- early determination of species or sex differences
- estimation of dose level for *in vivo* studies.

## Assay Principle

The assay is performed in a 96-well Teflon dialysis unit. Each well consists of 2 chambers separated by a vertically aligned dialysis membrane of pre-determined pore size. Plasma spiked with the compound of interest is added to 1 chamber and buffer to the other chamber. Over time, free compound diffuses from the plasma chamber to the buffer chamber until an equilibrium is reached. The unbound fraction is calculated as the concentration in the buffer side divided by the total concentration in the plasma side.

## Assay Protocol



## Typical Results

### Comparison of 4 Compounds using Pooled Human Mixed Gender Plasma

| Compound    | % Bound | Standard deviation | n | % Bound literature* |
|-------------|---------|--------------------|---|---------------------|
| Propranolol | 86.5    | 1.76               | 3 | 87 ± 6              |
| Diazepam    | 97.2    | 0.315              | 3 | 98.7 ± 0.2          |
| Digoxin     | 18.9    | 5.14               | 4 | 25 ± 5              |
| Warfarin    | 99.0    | 0.0789             | 3 | 99 ± 1              |

\* Goodman and Gilman's Pharmacological Basis of Therapeutics, 10<sup>th</sup> edition

### Species Comparison using Pooled Male Plasma

| Compound    | Species    | % Bound | Standard deviation* |
|-------------|------------|---------|---------------------|
| Propranolol | Human      | 85.8    | 1.24                |
|             | Beagle dog | 73.0    | 1.10                |
|             | Rat        | 79.3    | 1.36                |
| Warfarin    | Human      | 98.9    | 0.0425              |
|             | Beagle dog | 96.2    | 0.0521              |
|             | Rat        | 99.5    | 0.0154              |

\* Samples collected in triplicate