TRANSIL^{XL} Microsomal Binding Kit

A Fast High-Throughput Assay for Microsomal Binding

FEATURES AND BENEFITS

- Fast, requires only 20 minutes total assay time
- Measures the affinity to human microsomal membranes and determines microsomal binding
- Ready-to-use format in 96-well plate format generating highly reproducible results
- Rapid compound quantification due to immoblized brain membranes
- Kit includes a spreadsheet for calculation of final results and traffic light system for data quality rating

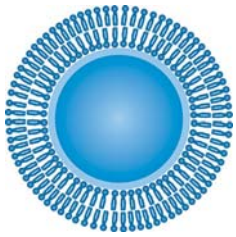

Fig. 1: Illustration of a TRANSIL Microsomal Binding bead with a single lipid bilayer reconstituted from synthetic lipids resembling a natural composition of human liver micromes.

TECHNICAL DESCRIPTION

The TRANSIL^{XL} Microsomal Binding kit measures the affinity of drugs to human microsomal membranes and determines microsomal binding in stability incubation experiments. This allows the accurate estimation of intrinsic clearance from stability incubations by correcting the experimental clearance with the fraction of drug unbound in the incubation.

The kit consists of ready-to-use 96-well microtiter plates. One plate can be used for measuring microsomal binding of up to 12 compounds. The assay requires only 5 steps: (i) addition of drug candidate, (ii) mixing and incubation for 12 minutes, (iii) removal of beads by centrifugation, (iv) sampling of supernatant, and (v) quantification of drug candidate.

CAPABILITIES

- Detection systems
  - LC/MS/MS
  - Scintillation counting
  - Others
- Parameters estimated and predicted
  - Affinity affinity to human liver microsomes
  - Human fu(mic)
  - coming soon: fu(mic) for other species

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Application and Relevance

Only free unbound compound is available to be metabolised by the enzymes present in microsomal incubations. Therefore, it is important to consider the extent of binding when performing microsomal clearance studies. Estimated incrinsic clearance rates can be substantially underestimated without correction for microsomal binding (fig. 2). Moreover, it has been shown that the prediction of in vivo compound stability improves substantially when correcting the metabolic rates obtained in microsomal incubations.

Microsomal binding not only reduces the concentration of free drug available to be metabolised by CYP enzymes, it also reduces the concentration which is available to inhibit the enzymes. It has been demonstrated that non-specific microsomal binding can account for underestimation of inhibitor potency (i.e., overestimation of IC_{50} or K_i values) when dealing with lipophilic basic drugs. This in turn can lead to an underestimation of risk of drug-drug interactions. In particular, mechanism based inhibitor studies can be affected to a large extent by microsomal binding, because of the high microsome concentrations that are typically employed in these experiments. Hence, the fraction of drug bound to microsomes is also an important correction of experiments assessing the inhibition potential.

Intrinsic Clearance

The intrinsic clearance rate (CL_{int}) is calculated from the observed clearance rate in the microsomal incubation experiment (CL_{obs}) by correcting for microsomal binding according to the following formula:

$$CL_{int} = \frac{CL_{obs}}{f_u(mic)}$$

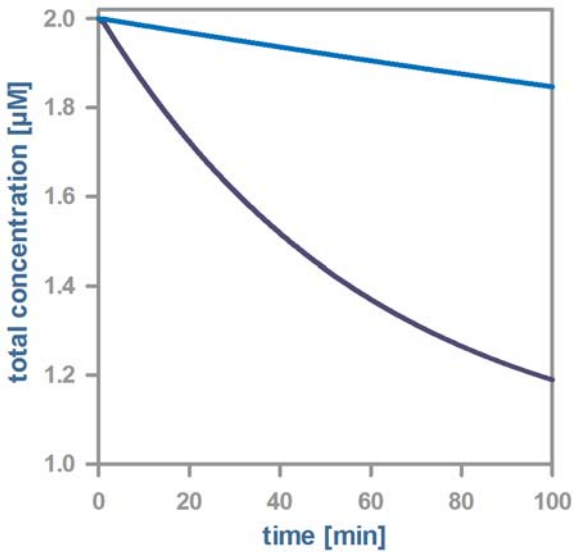


Fig. 2: Illustration of the influence of microsomal binding on clearance rate estimation. Both lines represent compounds with identical clearance rate. However, the compound represented by the dark blue line does not bind to microsomes, while the compound represented by the light blue line binds strongly $f_u(mic)$=10%. Hence, the intrinisc clearance rate of the compound binding to microsomes is highly underestimated.

Validation

A test set of 24 compounds was chosen for validation. Microsomal binding was measured using the TRANSIL^{XL} Microsomal Binding kit and conventional dialysis with microsomes (fig. 3). Both methods yield comparable results which correlate strongly (r^2=0.93).

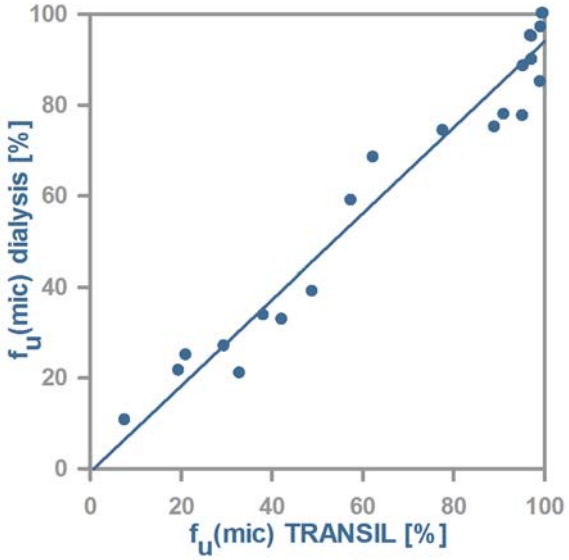
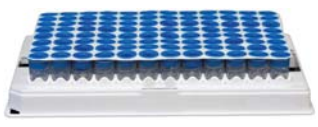


Fig. 3: Comparison of microsomal binding measurements using the TRANSIL Microsomal Binding Kit and conventional dialysis. The test set comprised alprenolol, amantadin, buspirone, carbamazepine, cyclobenzaprine, desipramine, diphenhydramine, Enalaprilat, fexofenadine, fluoxetine, fluvoxamine, faloperidol, ketoconazol, labetalol, levofloxazine, nalidixic acid, nortriptyline, promazine, propranolol, sulfasalazine, terazosin, venlafaxine, and warfarin.

PRODUCT INFORMATION

Order Number	Name
TMP-0120-2096	TRANSIL^{XL} Microsomal Binding kit



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