

TRANSIL^{XL}

Brain Absorption PLUS Kit

A Fast High-Throughput Assay for Brain Tissue Binding, Plasma Binding, and Brain Disposition

FEATURES AND BENEFITS

- Fast, requiring only 20 minutes total assay time
- Relevant. Measures brain tissue binding and plasma protein binding and predicts blood-brain barrier penetration
- Ready-to-use format in 96-well plate. Kit requires only addition of test material, 12-minute incubation, and quantification.
- Reliable with highly reproducible results, and robust correlation to dialysis
- Designed for automated liquid handlers. Alternative formats for manual pipetting available.

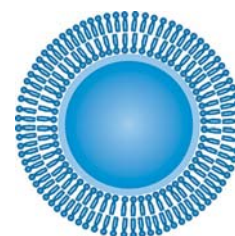


Fig. 1: Illustration of a TRANSIL Brain Absorption bead with a single lipid bilayer reconstituted from porcine brain lipids.

TECHNICAL DESCRIPTION

The TRANSIL^{XL} Brain Absorption PLUS kit estimates the binding of drugs to brain tissue and to human plasma. Also, the assay kit predicts the disposition of drugs into brain. It measures the affinity of drugs to reconstituted porcine brain membranes as well as the affinity of the drugs to physiological mixture of immobilized human serum albumin (HSA) and α_1 -acid glycoprotein (AGP). The brain membrane affinity is used to estimate the brain tissue binding and to predict the brain-to-plasma distribution via a hybrid model that also incorporates the drug's polar surface area and its plasma protein binding. Plasma protein binding is predicted from the affinity measurement to two major binding proteins HSA and AGP.

The kit comes in ready-to-use 96 well microplates. One plate can be used for measuring brain tissue binding, brain disposition, and plasma protein binding of up to 8 compounds. The assay requires on X 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
 - HPLC/UV
 - others
- Unbound fraction of drug in brain
- Unbound fraction of drug in plasma
- Brain-to-plasma distribution
- Passive diffusion across the blood-brain barrier

CORRELATION WITH DIALYSIS AND IN VIVO DATA

A strong correlation ($r^2=0.93$, $n=65$) is observed between the brain free fraction obtained with equilibrium dialysis and brain free fraction determined using the TRANSIL^{XL} Brain Absorption PLUS kit (fig. 2). The compound set represents a wide range of chemical structures with a range of logP from 0.6 to 6.2, a range of polar surface area from 3 to 26, and a range of H-bond donors from 0 to 4 and brain free fractions from 0.05% to 63%. Hence, the TRANSIL method yields comparable results to equilibrium dialysis. Likewise, a good correlation ($r^2=0.86$, $n=36$) between the predicted brain-to-plasma distribution coefficient (logBB) and the in vivo determined logBB was observed (fig. 3).

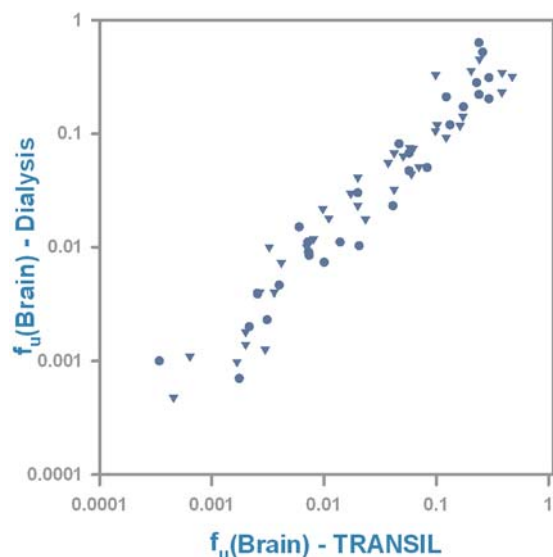


Fig. 2: Correlation of brain tissue binding estimates obtained from the TRANSIL^{XL} Brain Absorption PLUS kit and equilibrium dialysis ($r^2=0.93$).

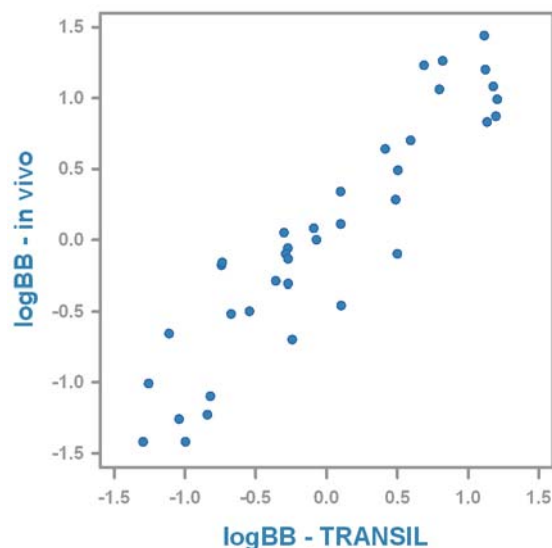


Fig. 3: Correlation of brain disposition estimates obtained from the TRANSIL^{XL} Brain Absorption PLUS kit and from *in vivo* experiments ($r^2=0.86$).

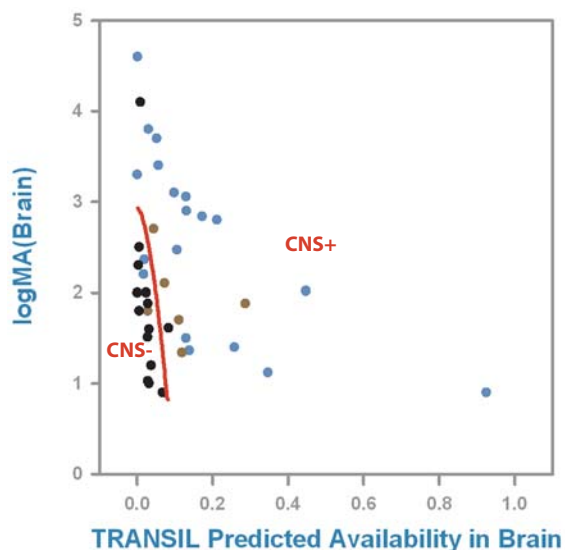


Fig. 4: Classification of CNS (blue), non-CNS (black) and drugs with CNS side effects (brown) using the TRANSIL^{XL} Brain Absorption PLUS kit using a computed classifier (red line)

CLASSIFICATION OF CNS VS NON-CNS DRUGS

Rate and extent are considered critical for brain penetration. The rate can be estimated by the log transform of the affinity to brain membranes (logMA). The best estimate for the extent is the free concentration of a drug candidate in the brain. The free concentration of a drug in brain is proportional to the product of the B/P ratio and the drug's free fraction in the brain, called brain availability. Using the rate and extent estimates from the TRANSIL Brain Absorption kit yields a reliable classification of compounds suitable as CNS drugs (CNS+) and compounds primarily targeting other organ systems (CNS-) compounds (figure 4).

PRODUCT INFORMATION

Order Number	Name
TCK-0200-1096	TRANSIL ^{XL} Brain Absorption PLUS kit