



TRANSIL® Product Range
Human Hepatocytes
Our Services

TRANSIL®

Brain Absorption Kit



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Significance of Brain Absorption

Early assessment of compound availability in the central nervous system (CNS) is essential for CNS drugs and useful for optimizing the toxicity profile of non-CNS drugs. Designing pharmaceutical agents so that they pass the blood-brain barrier and are freely available to interact with receptors is one of the great challenges in CNS drug development. The TRANSIL[®] Brain Absorption assay kit has been developed to help overcome this hurdle. Since the TRANSIL Brain Absorption Kit is a fast high-throughput format assay, it can also be used to assess brain penetration of any non-CNS drug in secondary screening. Hence, the assay helps to screen for compounds which are less likely to cause undesirable side effects in brain.

Significance of Brain Free Fraction

With increasing lipophilicity compounds tend to penetrate the brain more easily. However, since brain dry mass is mostly lipids, their unspecific binding in brain increases as well. This in turn, decreases the compounds' free fraction in brain. Hence, to determine the extent of brain penetration it is not only important to know how much compound enters the brain, but also how large the fraction of a compound is, that is freely available in brain. As brain membrane affinity correlates strongly with the brain free fraction estimate from dialysis against brain homogenate (figure 3), TRANSIL[®] Brain Absorption assay provides an accurate estimate of the brain free fraction.

Assay Principle

TRANSIL[®] Brain Absorption assay plates are ready-to-use. They are delivered with assay buffer and TRANSIL[®] beads with reconstituted porcine brain lipid membranes (figure 1). First, test articles are added in the same concentration (0.1 to 5 μM) to each well. After short mixing and incubation for 2 minutes (figure 2), TRANSIL[®] beads are easily separated by low-speed centrifugation. This allows the quantification of the remaining compound concentration in the supernatant. The measured brain membrane affinity is used directly to predict the brain free fraction. The brain-to-plasma distribution coefficient is predicted based on the measured membrane affinity, the compounds' calculated polar surface area, and the plasma protein binding.

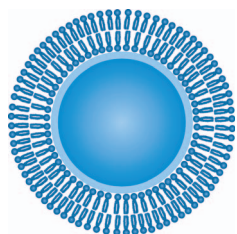


Figure 1: Illustration of a TRANSIL Brain Absorption bead with a single lipid bilayer reconstituted from porcine brain lipids.

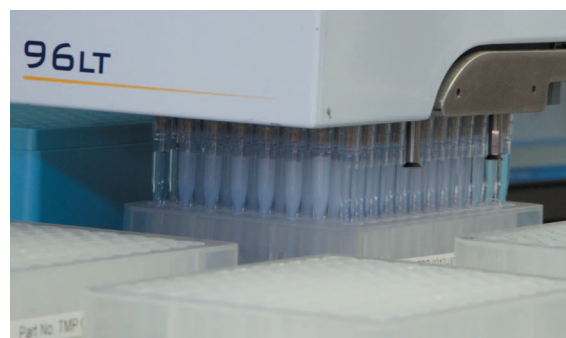


Figure 2: Fully automated use of TRANSIL Brain Absorption assay plates in Agilent's pipetting station. All liquid handling steps including mixing and spinning can be automated.

TRANSIL® Brain Absorption product description

Development of CNS drugs requires knowledge of the lead compounds ability to cross the blood-brain barrier. Frequently, this knowledge is also desirable in other therapy areas to prevent side effects. The TRANSIL® Brain Absorption kit is designed to assess drug candidates' ability to cross the blood brain barrier. The kit directly assesses test article's affinity for the brain's membrane. Membrane affinity is related to permeation (rate) and blood-to-brain absorption of a drug (extent). Also, the kit is used to predict the brain free fraction (figure 3). In combination with plasma protein binding data and polar surface area the model predicts the brain-to-plasma distribution coefficient \log_{BB} (figure 4).

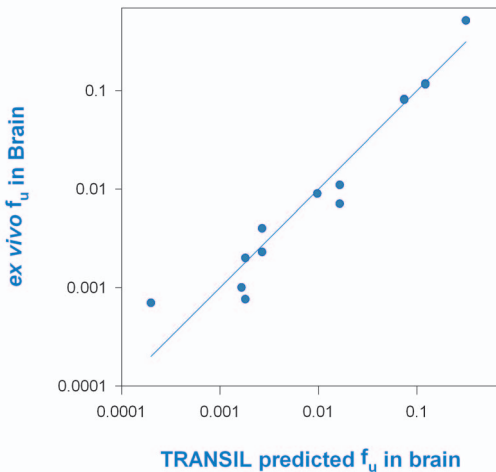


Figure 3: The brain free fraction predicted from the TRANSIL Brain Absorption kit correlates strongly ($r^2=0.94$) with the free fraction estimate from dialysis against brain homogenate.

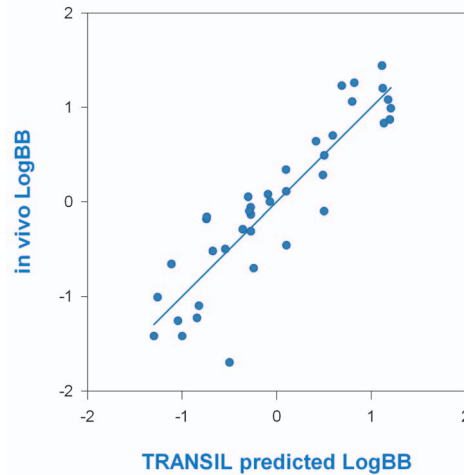
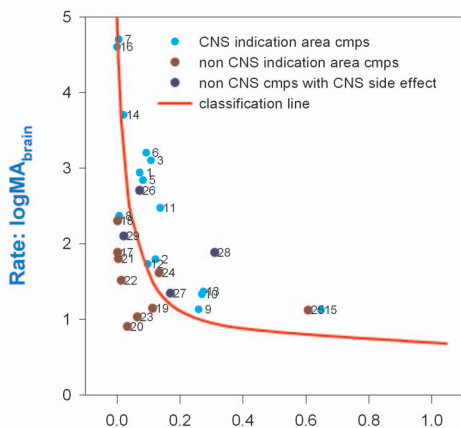


Figure 4: The brain-to-plasma distribution coefficient \log_{BB} predicted from the TRANSIL Brain Absorption kit correlates strongly ($r^2=0.86$) with *in vivo* data.

Compound Classification based on the TRANSIL Brain Absorption Kit

Rate and extent are considered critical for brain penetration. The rate can be estimated by the log transform of the affinity to brain membranes (\log_{MA}). The best estimate for the extent is the free concentration in brain. The free concentration in brain is proportional to the product of the B/P ratio and the brain free fraction, called brain availability. Using the



Extent: TRANSIL Predicted Brain Availability

Figure 5: Classification by rate and extent based on measurements with TRANSIL Brain Absorption kit. In this example 86% of the set of CNS and non-CNS drugs were correctly classified.

rate and extent estimates from the TRANSIL Brain Absorption kit yields a high performing classification of CNS+ and CNS+ compounds (figure 5).

Key to Compounds

1 Amitriptyline	17 Chlorambucil
2 Carbamazepine	18 Indomethacine
3 Desipramine	19 Nalidixic acid
4 Diphenhydramine	20 Atenolol
5 Imipramine	21 Cimetidine
6 Promazine	22 Ranitidine
7 Trifluoperazine	23 Tolbutamide
8 Verapamil	24 Pindolol
9 Acetaminophen	25 Procaine
10 Antipyrin	
11 Propofol	
12 Thioperamide	
13 Caffein	26 Propranolol
14 Chlorpromazine	27 Theophylline
15 Eserine	28 Pyrilamine
16 Thioridazine	29 Quinidine

Available Products and Services

The TRANSIL® Brain Absorption kits is available as ready-to use 96 well microplate, in 96 tubes format, or in solution according to the number of compounds that need to be analyzed. The following tables list the available products and services.

Order Number	Name	Format	Format
TMP-0110-0096	Transil® Brain Absorption Kit	Plate	96 Well
TMP-0110-1196	Transil® Brain Absorption Kit	Tube Unit	96 Tubes
TMP-0110-0025	Transil® Brain Absorption Solution	Solution	25 ml
TPB-0210-0096	Transil® HSA Binding Kit	Plate	96 Well
TPB-0210-1196	Transil® HSA Binding Kit	Tube Unit	96 Tubes
TPB-0210-0025	Transil® HSA Binding Solution	Solution	25 ml
TPB-0211-0096	Transil® AGP Binding Kit	Plate	96 Well
TPB-0212-1196	Transil® PPB Binding Kit	Plate	96 Well
TPB-0211-0025	Transil® AGP Binding Solution	Solution	25 ml

Order Number	Name
CST-0113-0001	Prediction of logBB, brain free fraction and plasma protein binding
CST-0110-0001	TRANSIL Brain Assay
CST-0210-0001	TRANSIL HSA Binding Assay
CST-0211-0001	TRANSIL AGP Binding Assay
CST-0212-0001	TRANSIL PPB Assay
CST-1001-0001	LC/MS Method Development

Literature

Boriss, H.; Tsaiun, K.; Annand, R. (2009): Evaluation of in vitro parameters to predict the unbound concentration in brain. Drug Metabolism Reviews 41(S1): 41.

Loidl-Stahlofen, A. et al. (2001): Multilamellar liposomes and solid-supported lipid membranes (TRANSIL): Screening of lipid-water partitioning towards a high-throughput scale. Pharmaceutical Research 18 (12):1782-1788.

