TRANSIL ASSAY KITS

Frequently Asked Questions (FAQs)

1.	What is the principle of **TRANSIL** technology?	The **TRANSIL** technology is based on surface-modified silica beads where proteins are covalently bound or membranes are non-covalently adsorbed to the beads surface with minimum non-specific bindings to the surface.
2.	What **membrane** is on the surface of the beads?	Beads carry **true phopholipid bilayers** of natural membrane composition, which mimic the true membrane properties such as fluidity and molecular interactions.
3.	What are the **key advantages** of the TRANSIL technology?	The TRANSIL products are based on **natural materials** reconstituted in a highly defined manner to offer unsurpassed reproducibility, while directly measuring the key parameters of interest.
4.	How **fast** are the TRASNSIL assays?	The TRANSIL assay incubation time is only **2 minutes**. The beads in one 96-well plate have roughly the surface area of a tennis court. This allows very rapid reaction and detection of protein binding or membrane permeability.
5.	What is the **procedure** for performing the TRANSIL Assay Kits?	A shared feature of all TRANSIL assays is the titration of a constant concentration of the test substance (2 - 50 μM) with different surface areas of lipid or protein, which is achieved by different amounts of TRANSIL beads per well. Two blank wells are used to determine non-specific binding to the assay plate. Thus the estimates of binding or permeability are not confounded by compounds sticking to the plate's plastic walls.
6.	What is the typical assay **set-up**?	The TRANSIL plates or tubes are supplied with different amounts of TRANSIL beads. After addition of the compound, mixing and incubation for 2 minutes, beads are separated by low speed centrifugation (5 minutes, 750 g). A fixed volume of the final supernate is transferred into a new blank plate for quantitation by UV, HPLC or LC/MS.
7.	What is the **optimal** concentration of compounds used for TRANSIL Assays?	It is recommend that compounds be used at concentrations of 2 -70 μM per well. A compound concentration of 50 μM is a good starting point for UV detection. Higher concentrations may cause interference and biased binding or permeability estimates. The lower binding limit of compound concentrations is only limited by the sensitivity of the detection system.
8.	What are the **TRANSIL Binding Kits**?	The TRANSIL Binding Kits contains beads coated with a **specific plasma protein** allowing the determination of the bound and free fractions of a pharmaceutical drug. The beads surface has been modified to minimize the non-specific bindings.
9.	Which TRANSIL **Binding assays** are available?	**Three types** of TRANSIL protein binding assays are available: Human Serum Albumin (HSA), α1-Acid Gylcoprotein (AGP) and Rat Serum Albumin (RSA).
10.	What is the **buffer** system in the TRANSIL Kits?	TRANSIL beads are suspended in **PBS buffer** (10 mM phosphate buffer, 155 mM NaCl, pH 7.4).
11.	How are the **proteins oriented** on the surface of the beads?	The proteins are randomly oriented on the surface of the beads through covalent immobilization procedure while preserving the protein binding sides fully functional.

12.	In what **formats** are the TRANSIL Kits available?	The standard TRANSIL Kits are in 96-well plate format. The intestinal absorption and HSA binding kits are offered in both 96-well format as well as 96-single tube format. Customized orders for i.e., 384-well plates are also offered. Please inquire toll free at 1 (877) 236-3235.
13.	**How many** determinations can be performed with the TRANSIL Kits?	The 96-well plate format allows for determination of **12** pharmaceutical drug candidates, using 8 wells per candidate drug. Two wells are used as the references and six wells for the drug candidate added to various concentrations of pre-dispensed beads in the wells.
14.	How does the TRANSIL **intestinal absorption** compare with the octanol/water partitioning technique?	The TRANSIL membrane beads predict permeability of both **ionized and non-ionized** compounds and compare well with permeation into liposomes, the current standard in permeability studies. In octanol/water partitioning, the permeation of small molecules through membranes depends on structural interactions (hydrogen binding Van der Waals) between the test substance and the membrane. Since octanol is structurally very different from phospholipids bilayers, it cannot model these interactions well. Also, octanol/water has shown to be particularly poor in predicting permeability of ionized compounds. Moreover, with an incubation time of only two minutes, the TRANSIL technology is more readily adaptable to **high throughput platforms** than the octanol/water partitioning.
15.	How do **TRANSIL** membrane permeability assays compare with **PAMPA** assays?	PAMPA assays use various unstructured lipid mixtures on filter membranes. Since these lipids do not have the structure of true phospholipids bilayers, they cannot mimic the structure and charge interactions between the test substances and the membrane nearly as well as the TRANSIL assays. Also non-specific binding confounds PAMA measurements more than the TRANSIL assays, since filter membranes are well-known to attract sticky compounds, which results in biased or even failed permeability estimates. Moreover, PAMPA assays require overnight incubation while TRANSIL assays require only two minutes incubation time. Finally, PAMPA plates have to be prepared and validated in your laboratory, while the TRANSIL plates are pre-validated and ready-to-use.
16.	Is it possible to use the TRANSIL beads in different **pH** environments?	Absorption of an orally administered drug is closely linked to the pH of the monitored compartment. The gastrointestinal fluid's pH differs vastly along the passage through the gastrointenstinal tract. Thus, total absorption and therefore bioavailability depends upon permeability rates of the full range of ionization stage of a drug. TRANSIL Intestinal Absorption Kits allow the determination of membrane permeability in a **pH** range of **3 - 11**. Thus, allowing estimation of membrane permeability across the full physiologically relevant pH range.
17.	Are TRANSIL beads available in other **buffers**?	All TRANSIL assays are supplied in PBS, pH 7.4. Please contact us toll free at 1 (877) 236-3235, if you require different buffer systems.
18.	What is the **stability** of the TRANSIL beads?	When stored at **-20°C**, the TRANSIL beads are stable at least for **6 months** after the delivery date.
19.	Is it possible to use **organic solvents** in the TRANSIL assays?	**DMSO,** when used at a concentration of **1%** as the solubility mediator causes no bleeding of the lipids or immobilized proteins. In general, pure organic solvents are not suitable for TRANSIL beads and detergents will affect the binding of the compounds to the binding sites of the plasma proteins.
20.	What type of **UV plates** should be used for the UV read-out?	For 96-well plates, the **Corning 96 well** half area plates **(No. 3679)**. Please check that the background absorbance above 250 nm is

		negligible for the UV quantitation.
21.	Can TRANSIL Assay Kits be **automated** using high throughput robots?	The TRANSIL assays can be **easily adapted** to automated platforms. The plates are compliant to SBS (Society of Biomolecular Screening) standards and are pre-filled with TRANSIL beads in PBS and ready-to-use. The assay protocol with 2 minutes incubation is readily adaptable to high throughput systems capable of low speed centrifugation (750 g). Examples include the TECAN Genesis, Zinser Lissy and Hamilton Microlab platforms.
22.	What are the **key issues** when using a high throughput liquid handler (automation)?	The set-up of an effective mixing device is essential to ensure **complete suspension of the beads** in the wells, as well as the exact programming of the **mixing velocities** to avoid cross contamination. After separation by centrifugation, the **immersion depth** plays an important role in avoiding displacement of the beads. Please refer to the product manual in the kit for setting up your liquid handler parameters.
23.	Can the plate, containing the supernates, after completion of the TRANSIL assay be **kept overnight** or longer?	Yes. To minimize evaporation during overnight incubation, plates should be covered with parafilm for a tight seal, keeping in mind that the compounds must be stable in PBS (the assay buffer).
24.	What are the prerequisites for using quantitation by **UV**?	Due to the background absorbance from most commercially available translucent plates and DMSO, quantitation by UV is only possible for compounds with significant absorbance above 250 nm. Also centrifugation of UV plates for 2 minutes at 750 g is recommended to remove any air bubbles that may cause light scattering.
25.	What is LogBB?	The logBB is the log of brain-to-plasma Distribution Coefficient, defined as: logBB = log (C [brain] / C [plasma]), where; C = Concentration of the compound of interest.
26.	What is the definition of **"availability"** for CNS and non-CNS compounds?	The **"availability"** is the product of the B/P ratio (which is the same as logBB, but not on the log scale) multiplied by the free fraction in the brain. Thus, the availability is the fraction of the compound that enters the brain and is available in the brain. The efficacy of the compound can then be obtained by dividing the availability of a compound by its receptor affinity (Ki). This would present the best scale for ranking compounds.
27.	What are the basic **physical characteristics** of the beads?	The beads are monodisperse silica beads and have a particle size distributions of about 10-13 µm in diameter.
28.	What is the volume of silica bead to the volume of dispersion?	The silica volume of the suspension is 1 minus correction factor (listed on the certificate of analysis) multiplied by the volume.
29.	Are **uncoated** beads available for sale?	Yes, **uncoated** beads are available as reference material, and can be ordered separately. Please call toll free at 1 (877) 236-3235 for more information.
30.	Can the beads be stored at **2-4°C**?	The beads can be stored at **2-4°C** for a short period of time (few hours). For long-term storage, the beads suspension should be aliquoted and stored at **-20°C.**
31.	Do the **Lipids** come off the beads during a long incubation period?	The **Lipids** usually do not come off during incubation. Thus the beads can be kept at RT for much longer time (i.e., 5 hrs). However, for such a long incubation time, the wells (or tubes) should not be vortexed, but instead rotated in order to provide a gentler mixing and keeping the beads in suspension during incubation. If any lipid is found on the surface of the assay buffer, this must have come from beads being damaged by extensive vortexing. During the product QC procedure we make sure that all beads are coated uniformly

		with the lipids.
32.	Why are **membrane permeability** and **protein binding** important parameters?	Membrane permeability affects drug absorption and protein binding affects its distribution in the body. Thus, both parameters are key indicators for bioavailability of drug candidates in the developmental pipeline.
33.	What is a **membrane affinity**?	Membrane affinity describes a small molecule's ability to permeate through lipid membranes. Therefore, it is directly related to the permeation rates across membranes such as intestinal membrane or the blood brain barrier.
34.	What parameters affect **absorption** across intestinal epithelium?	Total intestinal **absorption** depends on permeability across the intestinal epithelium as well as the drug's pH dependent solubility in the intestine and the passage time.
35.	What parameters affect **equilibrium distribution** of drugs between blood and brain?	**Equilibrium distribution** of drugs between blood and brain depends on the permeation across the brain's epithelial membranes, as well as the drug's protein binding.
36.	Can **water** be used for **washing** the beads?	1x dulbecco's PBS buffer system is recommended for **washing the beads**. Although the use of pure water is not expected to cause a problem, repeated centrifugation might cause an issue. Therefore, it is suggested that the beads be allowed to settle by gravity instead of centrifugation during the wash steps.
37.	How the TRANSIL Protein Binding Assay results **compare** with the plasma equilibrium dialysis results?	When comparing TRANSIL plasma protein binding with equilibrium dialysis using human plasma, different results can be expected due to following differences between the two approaches: • TRANSIL technology is based on purified proteins; therefore, all binding sites are expected to be available. Whereas, in plasma the binding sites may be masked; this may result in higher binding estimates by TRANSIL protein assay • TRANSIL protein assay measures the K_Ds to HSA or AGP or a pseudo- K_D to both (the combined assay) and calculates the binding based on assumptions of the absolute abundance of these proteins. The user can change these assumptions and adjust them to his/her own needs; however, they may not reflect the plasma composition chosen in the dialysis experiment. Thus, deviations of binding can go both ways depending upon the physiological and disease state of the plasma donor. • TRANSIL measures only HSA and AGP binding, which could cause underestimation of binding, since compounds could also bind to lipoproteins or other plasma proteins with low abundance.
38.	Why are **different amounts of beads** used in the TRANSIL assay?	In the TRANSIL kit format different concentrations of beads are used to cover a broad range of weak and strong binding compounds. Strong binders can tightly bind to beads in high concentration, so that there might be no compound left in the supernatant for detection. Weakly bound compounds may not bind strongly enough so that the differences in supernatants cannot be measured by the detection system (LC/MS). Therefore, different concentrations of beads are used to accommodate both scenarios. Moreover, the change in binding in response to change in protein concentration or membrane surface is used to calculate the binding constants.

39.	What is the recommended **compound concentration** for use in the TRANSIL Plasma Protein Binding Assays?	There is no lower limit to the recommended compound concentration. With the current state of the art LC/MS/MS, one could use 0.5 µM concentration for the assay. High compound concentrations can also be used, however, this may lead to saturation of individual beads and in the wells with fewer beads and to complete binding of the test compounds in the wells with many beads. However, this saturation clearly indicates strong binding; alas it cannot be exactly quantified. Only the intestinal absorption and HSA assay kits are robust against this kind of saturation. The recommended upper concentration limits for various TRANSIL Assays are; • 20 µM for the TRANSIL PPB and AGP kit • 50 µM for the TRANSIL Brain, Intestinal, and HSA kit
40.	What is the **saturation threshold** for lipid binding?	The saturation threshold of drugs on the beads has been set at 60 lipid molecules to 1 drug molecule. If there are more drug molecules bound in the membrane, then it is possible that the membrane structure is compromised, or altered in a way, such that the binding may behave differently. This threshold is based on assay design and probably has a 3-fold safety margin. Nothing is expected to happen to the binding when this ratio becomes lower.
41.	After thawing the kit's reagents, **how long** the reagents will be **stable** at 4°C?	We recommend that the plates be used within 24 h. Long term storage of the plates in the refrigerator is not recommended due to the particles aggregation.