# Science & Technology

## Frequently Asked Questions: TRANSIL Technology

1. **Why are membrane permeability and protein binding important parameters?**

   Membrane permeability affects drug absorption and protein binding affects its distribution in the body. Hence, both parameters are key indicators for bioavailability of drug candidates in the developmental pipeline. For effective and efficient drug development pharmacokinetic properties, drugs safety and efficacy need to be optimized at the same time. The products and services performed by Sovicell determine the pharmacokinetic parameters and distinguish potential safety problems before entering into a clinical trial.
   Membrane affinity describes a small molecules' ability to permeate through lipid membranes. Therefore it is directly related to the permeation rate across membranes such as the intestinal membrane or the blood brain barrier.
   Total intestinal absorption depends not only on permeability across the intestinal epithelium, but also on the drug's pH dependent solubility in the intestine and the passage time.
   Equilibrium distribution of drugs between blood and brain depend not only on the permeation across the brain's endothelial membranes but also on the drug's protein binding.

2. **What is the TRANSIL technology?**

   The core of the TRANSIL technology are silica beads with specific surface chemistry. The beads are modified such that we can covalently bind proteins or non-covalently bind membranes to their surface. Protein covered beads have very little exposed bead surface which itself has specific properties which prevents unspecific binding of test substances. Membrane beads carry true phospholipid bilayers of natural membrane composition. These membranes are reconstituted in our laboratory from natural materials and mimic precisely true membrane properties such as fluidity and molecular interactions.
   The key advantage of the TRANSIL technology is that the products are all based on natural materials that are reconstituted in a highly defined way in our laboratories, such that our assays offer unsurpassed reproducibility. At the same time they directly measure the key parameters of interest, since they are based on the natural materials of interest.

The beads in one 96-well plate have roughly the surface area of a tennis court. This allows very rapid detection of protein binding or membrane permeability. Hence, our assay kits require only two minute incubation time.

3. **What is TRANSIL for Protein Binding?**

   The TRANSIL Binding kits contain beads covered with a specific plasma protein allowing the determination of the bound and free fraction of a pharmaceutical drug. The surface was designed to minimize interactions with drug molecules, thus preventing non-specific binding. We offer three different types of proteins that are linked to the surface of the beads: Human serum albumin (HSA), alpha-1 acidoglyoprotein (AGP) and rat serum albumin (RSA).

4. **What is the buffer system of the TRANSIL kits?**

   TRANSIL beads are suspended in PBS buffer (10 mM phosphate buffer with 155 mM NaCl, pH 7.4).

5. **How are the proteins orientated on the surface?**

   The proteins are randomly orientated on the bead surface, and no loss of function due to the covalent immobilization procedure was observed.

6. **In what kind of format are the TRANSIL products available?**

   The standard format of our assay kits are 96-well plates. The TRANSIL Intestinal Absorption and the HSA Binding kit can be ordered both in a 96-well format or as 96 single tubes. Please contact us if you require customized formats such as 384-well plates.

7. **How many measurements are possible with the TRANSIL beads?**

   The 96-well format allows the determination of 12 pharmaceutical drug candidates, using 8 wells per drug candidate. We recommend the use of two references and six dilutions of the compound. The 96 tube format allows the determination of either membrane permeability or protein binding parameters with a minimum of only one compound.

8. **How does the TRANSIL intestinal absorption compare to octanol/water partitioning?**

Frequently membrane permeability is approximated by octanol/water partitioning. The drawback of this system is that permeation of small molecules through membranes depends on structural interactions between the test substance and the membrane such as hydrogen bonding Van der Waals interactions. Since octanol is structurally very different from phospholipid bilayers, it cannot model these interactions well. Octanal/water partitioning has been shown to be particularly poor in predicting permeability of ionized compounds. Our membrane beads are just as good at predicting permeability of ionized and non-ionized compounds and compare well to permeation into liposomes, which is regarded as the gold standard in permeability studies. Moreover, with an incubation of only two minutes our technology is more easily scalable to higher throughput than octanol/water or liposome/water partitioning.

9. **How do TRANSIL membrane permeability assays compare to PAMPA?**

   PAMPA assays use various unstructured lipid mixtures on filter membranes. Since the lipids do not have the structure of true phospholipid bilayers, they cannot mimic the structure and charge interactions between the test substances and the membranes nearly as well as the TRANSIL assay kits.
   Also, non-specific binding confounds PAMPA measurements more strongly than TRANSIL assays. Filter membranes are well known to attract sticky compounds. This interaction with the filter membranes results in biased or even failed permeability estimates.
   PAMPA assays require overnight incubation while TRANSIL plates require only 2 minutes incubation time.
   PAMPA plates have to be prepared and validated in your laboratory, while TRANSIL plates are ready to use and validated by us.

10. **Are protein binding and membrane permeability measurements available as a service?**

    We offer the determination of membrane permeability and protein binding in a fee-for-service model. We also offer pharmacokinetic modeling to predict a large number of important kinetic parameters. Our service unit not only offers assays based on our in-house technology but also industry standard assays covering a broad range of parameters. In case of strong protein binding we perform the distribution assay according to the procedure of Schuhmacher by using human erythrocytes in our High Protein Binding Assay.

11. **Is it possible use TRANSIL beads in different pH environments?**

    Absorption of an orally administered drug is closely linked to the pH value of the monitored compartment. The gastrointestinal fluid's pH differs vastly along the passage through the gastrointestinal tract. Hence, total absorption and thus 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 range from pH 3 to 11. Thus, the TRANSIL technology allows estimation of membrane permeability across the full physiologically relevant pH range.

12. **Are TRANSIL beads also available in other buffer systems?**

    Generally all assays are performed in PBS at the pH of 7.4. Please contact us if you require different buffer systems.

13. **How stable are TRANSIL beads?**

    Stored at -20 °C the TRANSIL beads are stable for at least 6 months after delivery.

14. **What is the principle of TRANSIL assay kits?**

    A shared feature of all TRANSIL assays is the titration of a constant concentration of the test substance (2 to 50 µM) with different surface areas of lipid or protein, which is achieved by different amounts and sizes of TRANSIL beads per well. Two blank wells are used to determine unspecific binding to the assay plate, hence our estimates of binding or permeability are not confounded by compounds sticking to the plate's plastic walls.

15. **What is the typical assay set-up?**

    The plates or the tubes are delivered with the different concentration of TRANSIL beads. After compound addition, mixing and incubation for 2 minutes, beads are separated by low speed centrifugation (5 minutes with 750 g). A fixed volume of the final supernatant is transferred into a new plate for quantification either by UV, HPLC or LC/MS.

16. **What is the final concentration of the compound in the assay?**

We recommend that compounds are used at final concentrations that range between 2 and 70 µM per well if possible. 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 bound of compound concentrations is only limited by the sensitivity of the detection system.

17. **Is it possible to use organic solvents in the assay?**

    TRANSIL beads are not suitable for use in pure organic solvents or in detergent solutions. However, solubility mediators like DMSO cause no bleeding of the lipids or immobilized proteins when used at low concentrations. We recommend a DMSO concentration of 1% for the assay conditions.

18. **Which kind of UV plates should be used for UV read-out?**

    For 96 well plates we recommend the Corning 96 well half area plates (No. 3679). Please check that the background absorbance above 250 nm is negligible for quantification.

19. **What are the prerequisites to use quantification by UV?**

    Quantification by UV is only possible for compounds with significant absorbance above 250 nm due to the background absorbance of most commercially available translucent plates and DMSO. We recommend centrifugation of UV plates for 2 minutes at 750 g to destroy any air bubbles that otherwise may cause light scattering.

20. **Are other assay configurations/formats possible?**

    In some cases it might be necessary to adapt the assay format/configuration to your specific needs. Please contact us to generate a solution.

21. **Can TRANSIL assay kits be handled by robots?**

    The TRANSIL assay kits can be easily used in an automated environment. The assay plates are compliant to SBS (Society of Bimolecular Screening) standards and are already filled with fixed volumes of buffer (PBS) and TRANSIL beads. They will be delivered in frozen and come ready-to-use. The assay starts with the test item addition in one pipetting step. After mixing and incubation for 2 minutes, the beads are separated by low speed centrifugation (5 minutes with 750 g). A fixed volume of

the supernatant or is transferred into a new plate for quantification by UV, HPLC, or LC/MS.

22. **What are the key liquid handling issues for automation?**

    An important point is the set up of the mixing step to ensure a complete re-suspension in the well, including the exact programming of the mixing velocities to avoid cross contamination. After separation by centrifugation the immersion depth plays an important role to avoid displacement of the beads. For the transfer of the supernatant the needles of the pipetting device should be placed at least 2 mm above the settled beads within the wells. Usually that is achieved by placing the needles at least 11 mm above the zmax-position within the wells, which is defined as the maximum immersion depth.

23. **On which automated platforms are the TRANSIL Kits transferable?**

    Our TRANSIL assay kits have been validated on a large variety of platforms including the TECAN Genesis, Zinser Lissy and Hamilton Microlab platforms.