Corning® BioCoat™ PAMPA Plate System

Frequently Asked Questions

CORNING

Specifications of the PAMPA Microplate

1. **What are the dimensions of the PAMPA plate system?**

Filter Plate (Acceptor Plate)	
Number of wells	96
Well shape	Round
Well bottom	Flat
Plate dimensions (L x W x H)	127.9 x 85.6 x 14.6 mm
Column offset left edge to A1 center	14.4 mm
Row offset top edge to A1 center	11.4 mm
Well spacing	9 mm
Well diameter	6.2 mm
Well height	12.1 mm

Receiver Plate (Donor Plate)	
Dimensions (L x W x H)	127.8 x 85.5 x 17.4 mm

2. **What membrane material is used in the filter plate?**

   Polyvinylidene fluoride (PVDF).

3. **What is the filter pore size?**

   PVDF membrane pore size is 0.45 µm.

4. **What lipids are used in the artificial membrane?**

   The artificial membrane consists of structured tri-layers of phospholipids.

5. **What material is used for the receiver plate?**

   Polystyrene.

6. **Is the receiver plate coated?**

   No.

7. **Is there an issue with non-specific binding on the receiver plate?**

   No issues have been encountered with assessing permeability due to non-specific binding on the receiver plate for the compounds tested. It is possible some compounds bind to the receiver plate non-specifically. The mass retention values for the compounds tested has been low, and low mass retention does not affect the ability to calculate permeability (the formula used for calculation has accounted for mass retention). The Corning® BioCoat™ PAMPA membrane reduced the mass retention of high mass retention compounds tested, such as amitriptyline, ketoconazole, and phenazopyridine when compared to traditional PAMPA. This demonstrates that a significant portion of mass retention for these compounds is contributed by the extra solvents in the traditional PAMPA membrane. Although it is possible the remaining portion of mass retention for these 3 compounds are contributed by non-specific binding to the plate, the permeability values for these 3 compounds can still be calculated without an issue.

8. **Can I use a portion of the Corning PAMPA plate in one experiment, and the rest of the plate in a second experiment on a different day?**

   No. The entire plate must be used in one experiment.

Membrane Stability, Integrity, Shipping, and Storage

1. **Are the Corning BioCoat PAMPA membranes stable after transportation (both domestically and internationally)?**

   Yes. The artificial membrane is stable when stored at -20°C and shipped with dry ice.

2. **If the plates are not properly stored in the freezer upon receipt and are thawed out, can they still be used?**

   The plates should be stored in the freezer immediately upon receipt. If the plates have been thawed out for more than 24 hours, they cannot be used.

3. **Can the package be opened before warming to room temperature?**

   Yes. When the plates are taken out of the freezer to warm up, the package can be opened at any time. Once the plates are warmed to room temperature, they must be used within the same day.

4. **Is an integrity test necessary or recommended after the assay?**

   No. It is advisable to use a few standard compounds in each PAMPA experiment to confirm the integrity of the membrane. The standard compounds should include at least one low permeability compound and one high permeability compound. If the correct ranking is obtained, this confirms the integrity of the membrane.

Preparation of Compound Solutions: Buffer, Concentration, Organic Contents, pH, Compatibility with other Chemicals

1. **What initial concentration of the compounds is typically used?**

   It depends on which method is used to measure compound concentrations. If LC/MS is used, lower concentrations such as 10-20 µM can be used.

   If a UV plate reader is used, 100-200 µM is recommended. If the UV absorption coefficient of a particular compound is very low, even higher concentration is recommended. It is recommended that the initial concentration have a UV absorption value at least 50 times higher than the detection limit of the UV plate reader. For example, if the detection limit for absorption is 0.001, then the initial concentration should have absorption >0.05. This ensures the concentration of the acceptor solution, which may be only a few percent of the initial concentration, can be measured. It is critical that a UV-transparent plate is used, such as the Corning 96-well clear flat bottom UV-transparent microplate (Corning Cat. No. 3635).

2. **What can I do if a compound has low solubility and the initial concentration is not high enough for concentration measurements?**

   It is recommended that 10% to 20% methanol is added to the PBS buffer to increase the solubility of the compound. High percentage of DMSO (>5%) is also compatible with the PAMPA membrane but it is not recommended, because a high percentage of DMSO interferes with UV absorption.

3. **What pH range is suitable for PAMPA membranes?**

   Usually PBS (pH 7.4) is used as the acceptor buffer. The donor solution buffer can be either PBS (pH 7.4) or a buffer with lower pH (such as pH 6 or pH 5).

4. **Can I use the pION "double-sink" buffer?**

   No. The artificial membrane is not compatible with the pION "double-sink" buffer due to the presence of surfactants in the buffer.

5. **Can I use bile acids or bile salts in the buffer?**

   No. The artificial membrane is not compatible with bile acids and bile salts. Bile acids and bile salts are surfactants that can dissolve the lipids in the artificial membrane.

6. **What is the lowest pH you have tested for the assay?**

   The lowest pH we have tested was 4.0, but pH 7.4 is optimal and will provide you with the best test results.

7. **Does "5% DMSO and 20% methanol compatibility" mean that compounds can be dissolved in DMSO and then diluted into 20% methanol/PBS at a pH of 5.0 or 7.4?**

   The compounds can be dissolved in DMSO and diluted into 10% methanol (20% is the limit when DMSO is not used) / PBS at pH 7, pH 6 or pH 5. A lower pH may be acceptable but has not been tested at Corning.

8. **The specifications say 5% DMSO or 20% methanol can be used. Have other solvents been tested? Has acetonitrile been tested, and in what concentrations?**

   Acetonitrile is fine with concentrations up to 10%. The membrane can tolerate up to 20% methanol or acetonitrile, however it is recommended to use 10% methanol or acetonitrile plus up to 2% DMSO. For low solubility compounds, we recommend diluting the DMSO stock solution of the compound into PBS+10% methanol or PBS+10% acetonitrile.

Experimental Setup: Temperature, Humidity, Agitation, Incubation Time, Plate Orientation

1. **Do the PAMPA plates need to be incubated in a humidified chamber?**

   No. Humidified chambers are not necessary for Corning® BioCoat™ pre-coated PAMPA plates due to the relatively short incubation time (4 to 5 hours). We compared results obtained with or without a humidity chamber and found no difference.

2. **What incubation temperature is recommended?**

   Room temperature is recommended. However, the artificial membrane is robust and higher incubation temperatures such as 37°C can be used. The permeability values will be higher compared to room temperature due to thermodynamic effects.

3. **Is agitation needed? Can I use shaking with the PAMPA plates?**

   No agitation is needed. However, the artificial membrane is robust and moderate plate shaking may be used. The permeability values will be higher compared to static incubation due to the agitation.

4. **Are the compound solutions supposed to be added to the receiver plate or the filter plate? What is the difference?**

   We recommend the compound solutions are added to the receiver plate (bottom plate) and the buffer is added to the filter plate (top plate). This method is used by many PAMPA researchers. If the compound solutions are added to the plates manually, it may take a longer time to prepare the compound donor plate with many different compound solutions, than to prepare the buffer acceptor plate with one buffer. Since all of the wells of the filter plate should be wetted at approximately the same time, it is better to add buffer to the filter plate, which is a relatively quick process, compared to adding many different compound solutions.

   However, the opposite method can also be used: the compound solutions are added to the filter plate (top plate) and the buffer is added to the receiver plate (bottom plate). This method is used by many Caco-2 researchers. The permeability values obtained with compound solutions on the top (Caco-2 convention) will be higher than the permeability values obtained with the compound solution in the bottom (PAMPA convention). The change of the absolute observed permeability values will not affect the ranking of the compounds into low and high permeability but different ranking criteria will need to be used:

   If the compound solutions are in the bottom receiver plate (PAMPA convention):
   Permeability <15 nm/s (or 1.5 x 10^{-6} cm/s) → low permeability
   Permeability >15 nm/s (or 1.5 x 10^{-6} cm/s) → high permeability

   If the compound solutions are in the top filter plate (Caco-2 convention):
   Permeability <40 nm/s (or 4.0 x 10^{-6} cm/s) → low permeability
   Permeability >40 nm/s (or 4.0 x 10^{-6} cm/s) → high permeability

   NOTE: Using the PAMPA convention makes it easier to compare the data with traditional PAMPA results, and using the Caco-2 convention makes it easier to compare Caco-2 results.

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