



# PAMPA Permeability Assay

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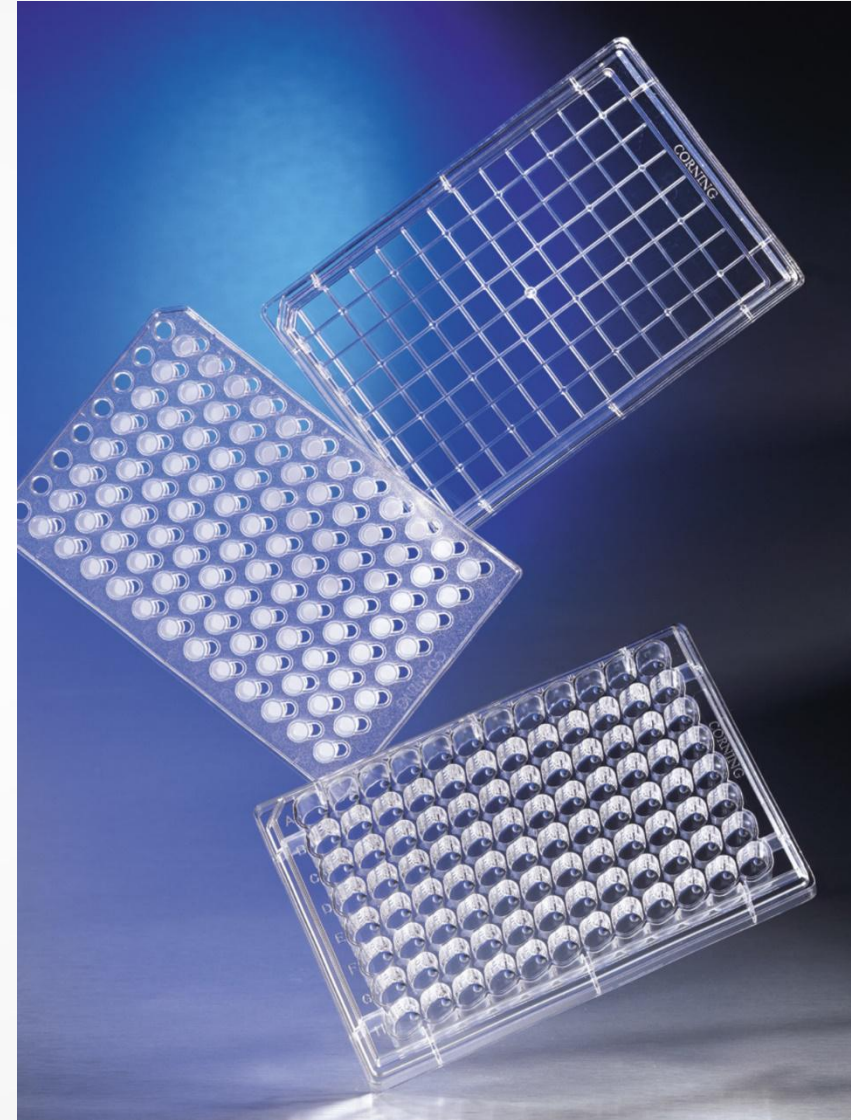
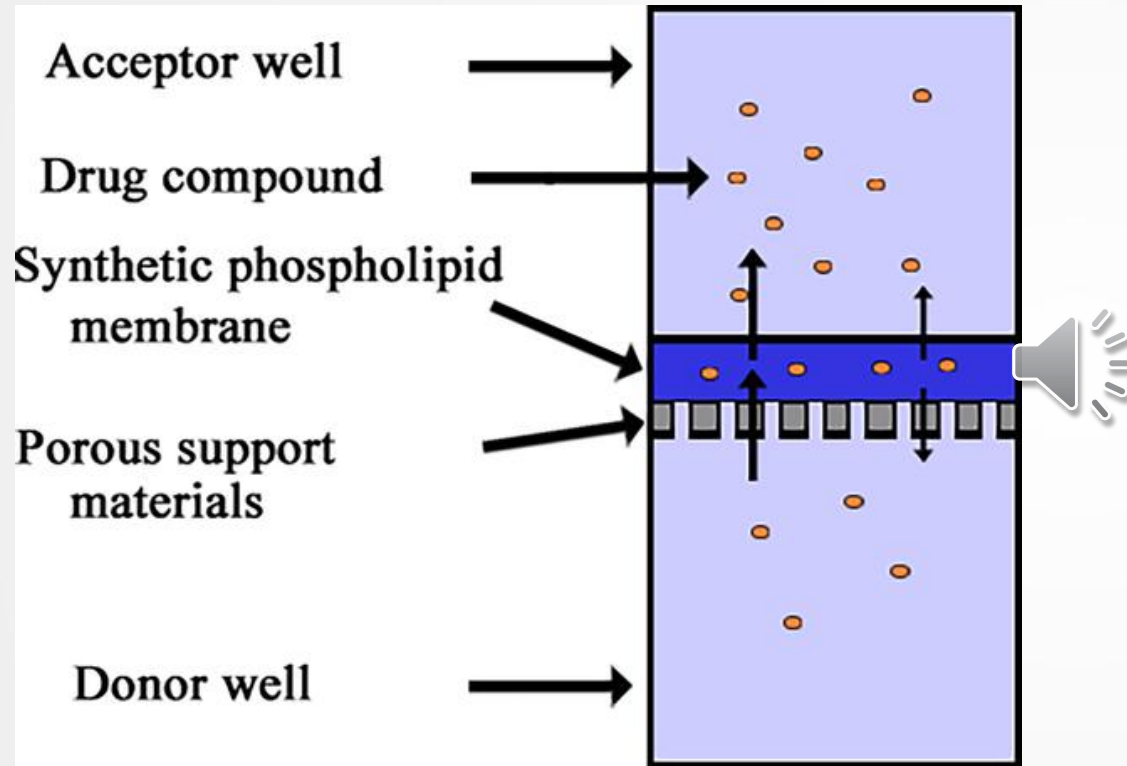
**PAMPA** is a method which determines the permeability of substances from a donor compartment, through a lipid-infused artificial membrane into an acceptor compartment.

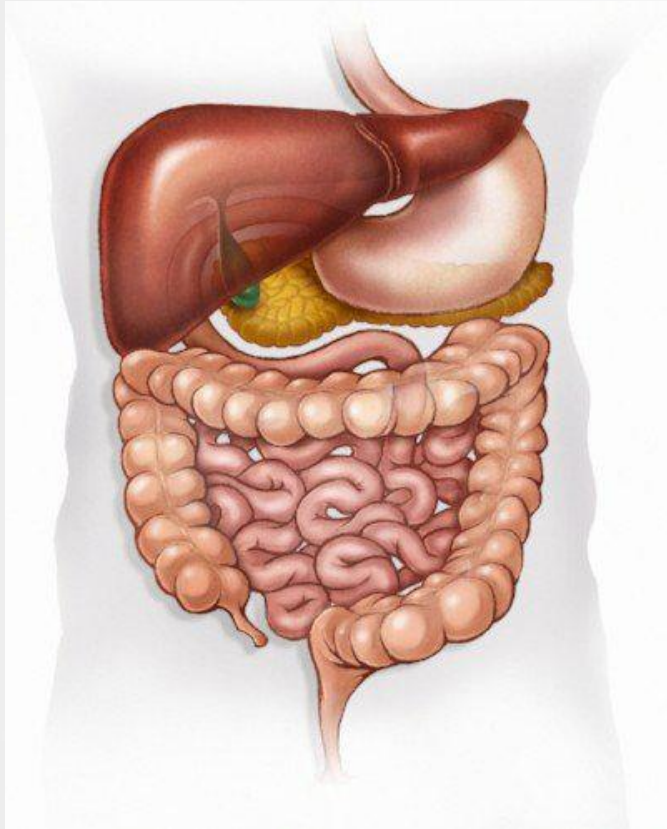


A multi-well microtitre plate is used for the donor and a membrane/acceptor compartment is placed on top; the whole assembly is commonly referred to as a "sandwich" .

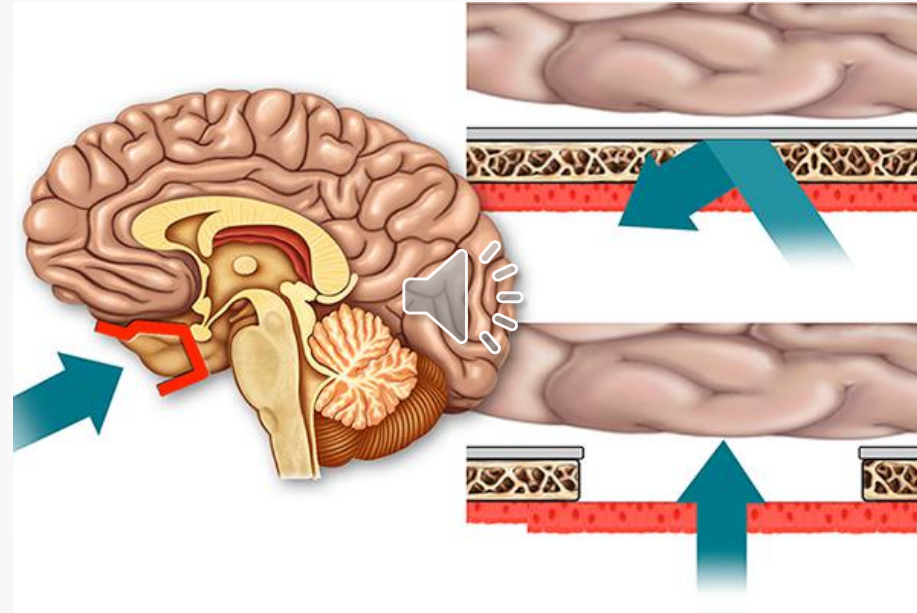


# Part 1 PAMPA Experimental Setup

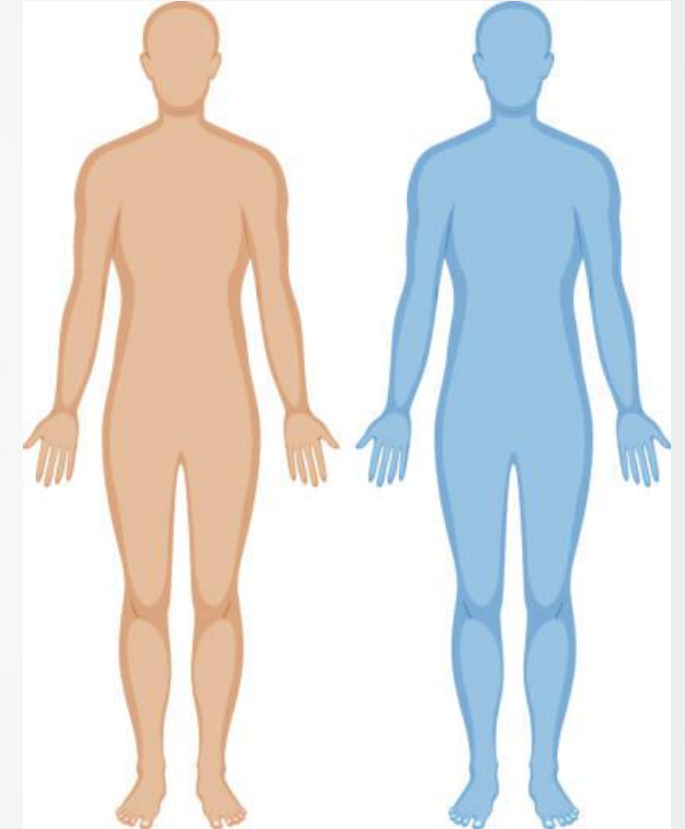




Gastrointestinal Permeability



Blood-Brain Barrier Permeability



Skin Penetration



## Advantages

PAMPA is cost-effective, easy to automate, and compatible for a high percentage of solubilizers. PAMPA has good day-to-day reproducibility and small variability. The cost of PAMPA is more than 10-fold lower than that of Caco-2 assay. The PAMPA membrane can be instantly prepared at the time of use, while Caco-2 requires a 2-3 days culture before use. PAMPA enables studies of passive transcellular permeation without intervention by paracellular and active transports.



## Limitations



One limitation of PAMPA is that the membrane is not exactly same as the biological membrane. The PAMPA membrane contains an organic solvent, possibly resulting in a non-bilayer membrane structure. In addition, PAMPA has neither active transport systems nor a paracellular pathway. The *in vivo* prediction accuracy of PAMPA is comparable to or less than Caco-2.

	<b>Caco-2</b>	<b>PAMPA</b>
<b>Type of model</b>	Cell monolayer	Artificial membrane
<b>Type of Permeability</b>	Measures the sum of passive and active permeability	Measures passive permeability in absence of transporters or efflux systems
<b>Assay preparation</b>	Requires cell culture (up to 21 days) Easy, fast preparation	Easy, fast preparation
<b>Cost</b>	High	Low

PAMPA and Caco-2 tests can be complementary tests to determine both passive and active permeability. Caco-2 tests alone measure the sum of passive and active permeability which can not be decoupled without the information obtained from PAMPA tests.

- 1 Original PAMPA**

The lipid solution consists of 10% lecithin in dodecane.
- 2 DOPC-PAMPA**

The lipid solution consists of 2% DOPC in dodecane.
- 3 HDM-PAMPA**

The lipid solution is 100% hexadecane.
- 4 •Bio-mimetic PAMPA (BM-PAMPA)**

The lipid solution consists of a mixture of PC, PE, PS, PI and cholesterol in an organic solvent.
- 5 Double-Sink™ PAMPA (DS-PAMPA)**

The lipid solution consists of 20% dodecane solution of a phospholipid mixture and the acceptor solution contains a surfactant mixture.

## Prepare solutions

- Prepare target analyte to a dilution of 1-10  $\mu\text{M}$  in a buffer of 1X PBS pH 7.4 and 5% DMSO.
- Prepare a 1% lecithin in dodecane solution in an Eppendorf tube, and sonicate until fully mixed.



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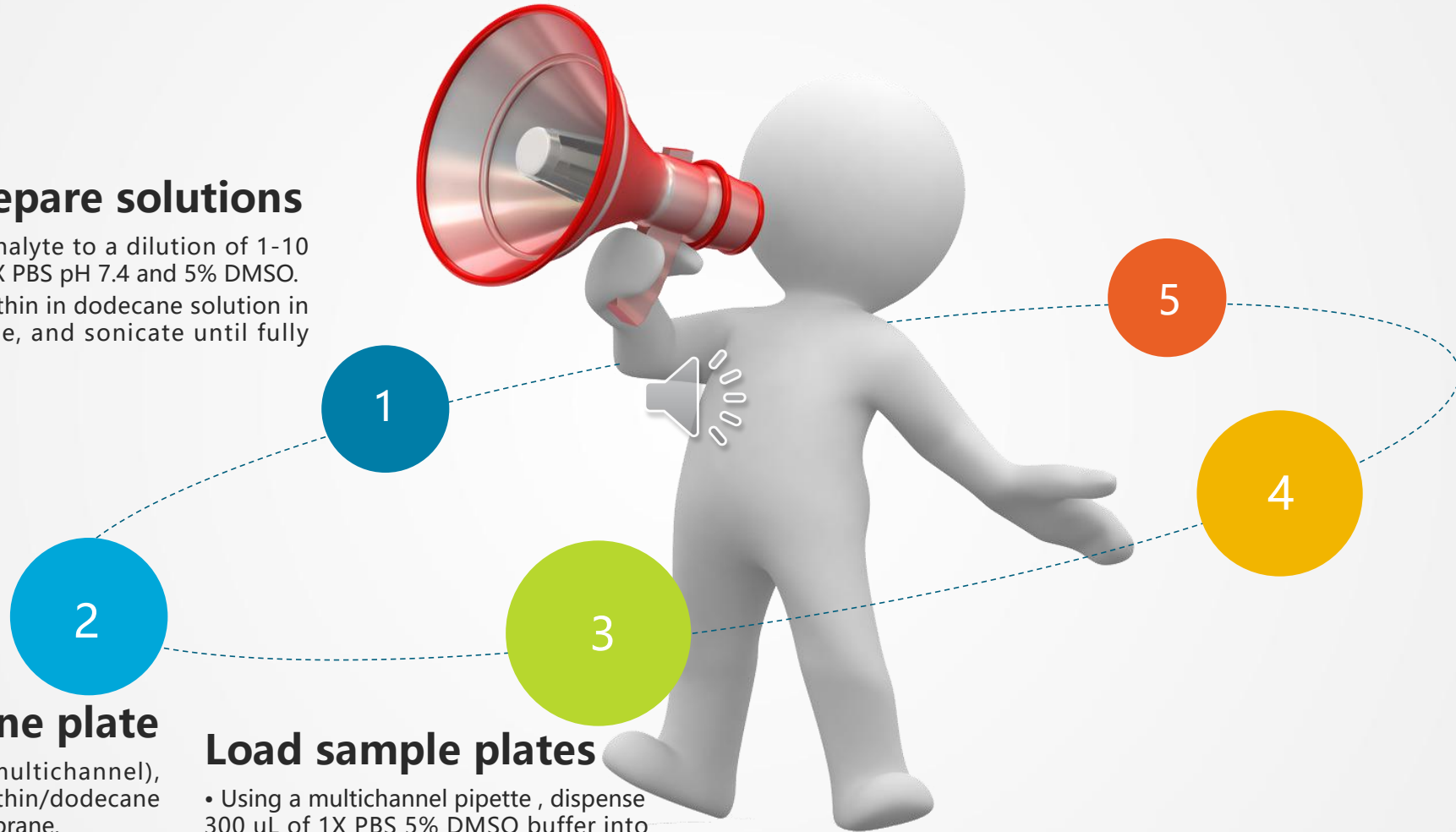


## Prepare membrane plate

- Using a pipette (single or multichannel), gently dispense 5 $\mu\text{L}$  of 1% lecithin/dodecane solution onto donor plate membrane.

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## Prepare membrane plate

- Using a pipette (single or multichannel), gently dispense 5  $\mu\text{L}$  of 1% lecithin/dodecane solution onto donor plate membrane.

## Load sample plates

- Using a multichannel pipette, dispense 300  $\mu\text{L}$  of 1X PBS 5% DMSO buffer into the acceptor plate wells, and transfer 150  $\mu\text{L}$  of the 10  $\mu\text{M}$  analyte into the donor plate wells.

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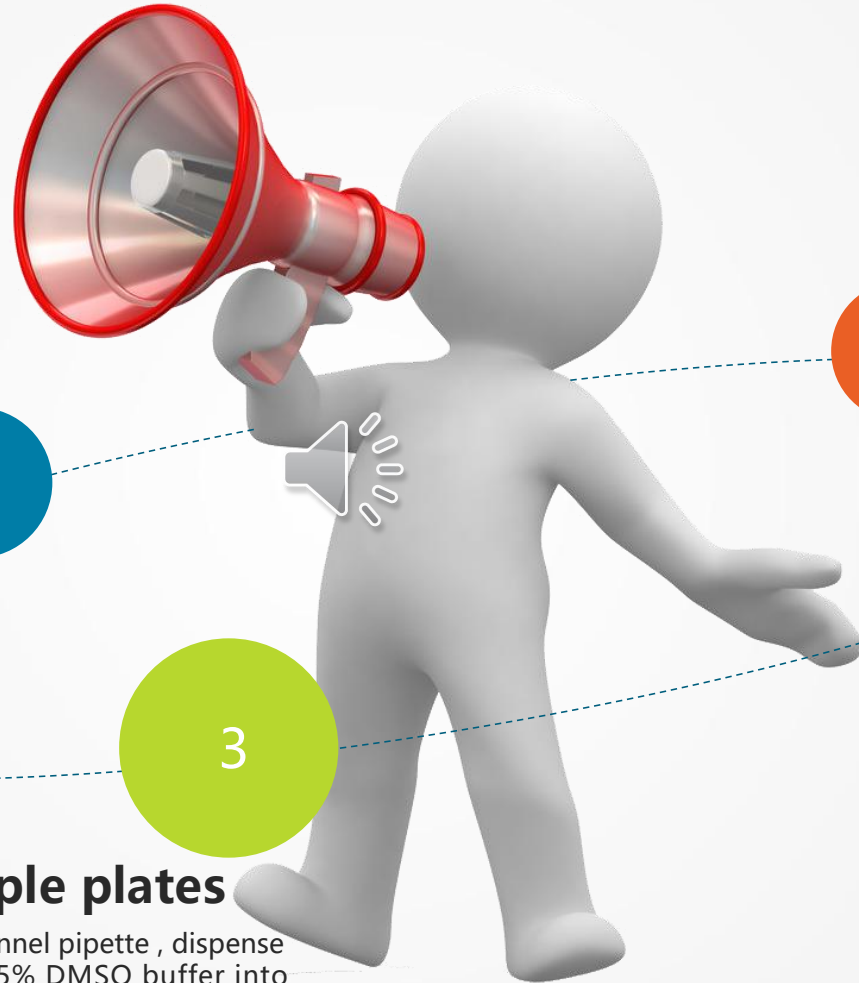
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## Run assay

- Gently transfer the assembly to a moist separate chamber.
- Allow assembly to sit for 10-20 hours.
- Transfer 100 $\mu\text{L}$  from each well to a labeled 96-well plate for HPLC-MS injection.

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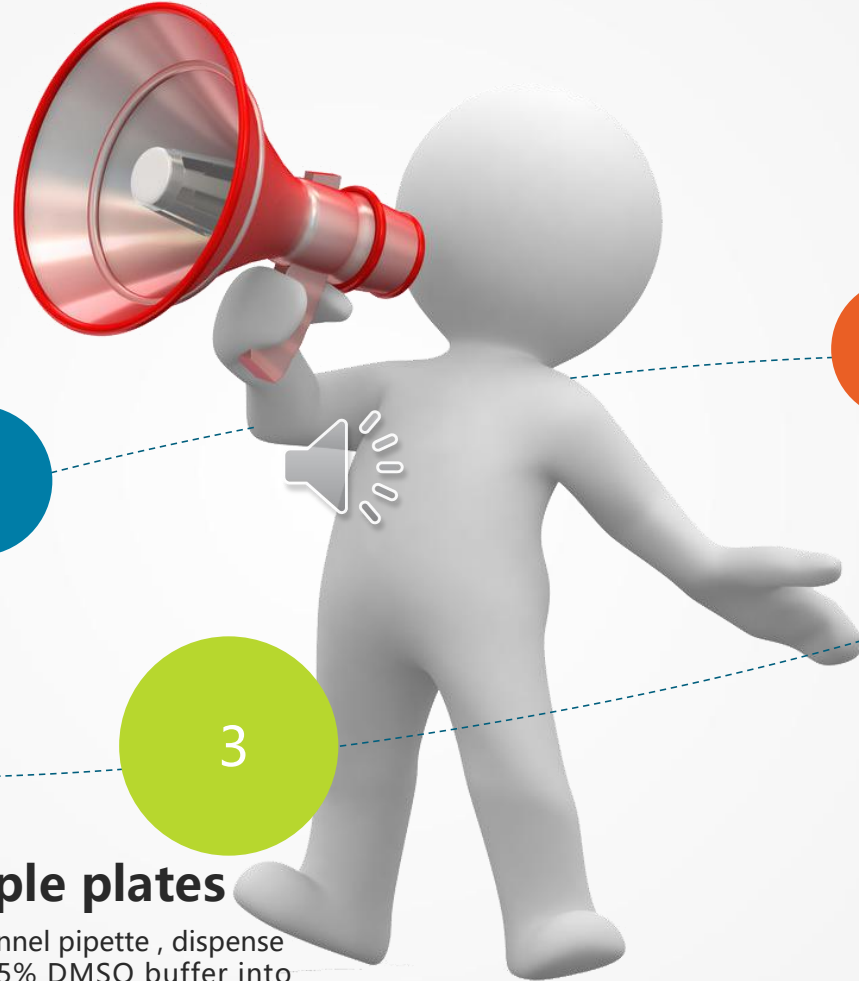
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## Analyze & Calculate

- Analyze all samples within 24-48 hours after running the assay.
- Create an appropriate HPLC-MS method.

4

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# THANK YOU

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