

## Product Information

### Unpacking Instructions – 24 Well Assay Ready Plate

#### Equipment Required but Not Provided.

Receiver tray not included and one must be purchased separately (Catalog Number PSMW010R5) for each Assay Ready Plate.

#### Procedure

The shipping medium must be changed on the day the plate is received. The unpacking instructions should be followed for each plate that is received.

#### Unpacking

Upon receipt, open the box and remove the plastic Ziploc® bag containing the Multiwell Insert – 24 well Assay Ready Plate.

#### Changing the Shipping Medium

1. On day of receipt, prepare everything needed to replace the shipping medium with fresh Caco-2 cell culture medium:
  - cell culture biosafety cabinet
  - standard Caco-2 medium, pre-warmed to 37 °C
  - aspiration system
  - 24 well receiver tray (one for each plate received)
  - sterile containers for culture medium
2. Remove the Multiwell Insert – 24 well Assay Ready Plate (still at room temperature) from the Ziploc® bag.
3. In a biosafety cabinet, unwrap the Multiwell Insert – 24 well Assay Ready Plate and carefully pull off the Parafilm®.
4. Open the Assay Ready Plate, placing the lid next to the plate, facing upwards. Carefully remove the ThermalSeal® film.
5. Add 400 µl of culture medium in each apical well.
6. Place the lid back on the 24 well Assay Ready Plate and place in the cell culture incubator for a minimum of 4 hours to allow the transport medium to liquefy.
7. Prepare the receiver tray for changing the medium:
  - a. Ensure the Caco-2 medium has been warmed to 37 °C

- b. In biosafety cabinet, unwrap one 24 well receiver tray (Catalog Number PSMW010R5, must be ordered separately) for each Assay Ready Plate. Place lid next to the plate, facing upwards.
- c. Add 800 µl of warm Caco-2 medium in each basal well.
- d. Place the lid on the plate and place the plate in the incubator.

**Notes:** Once the shipping medium has liquefied, proceed to step 8. The following must be performed using sterile technique in the biosafety cabinet.

Never handle more than one plate at a time while changing the shipping medium.

8. Take one Assay Ready Plate and one receiver tray out of the incubator and place both in biosafety cabinet.
9. Open the Assay Ready Plate, placing the lid next to the plate, facing upwards.
10. Gently lift the apical (upper) section of the Assay Ready Plate and place it on the new receiver tray.
11. Cover the receiver tray with its lid, then put it back into the cell culture incubator.
12. Repeat steps 1–11 for additional plates.

**Notes:** After the shipping medium has been changed to fresh Caco-2 medium, the plate should be kept in the incubator until day 21. Transwell assays can be performed on days 21–25.

Culture medium should be replaced every 48–72 hours.

#### Additional Product Information

For the complete Technical Bulletin, which includes procedures on TEER Measurement, the Transwell Assay, and the Lucifer Yellow Assay go to [www.sigma.com](http://www.sigma.com) and conduct a search for the respective Assay Ready Plate catalog number.

Ziploc is a registered trademark of S.C. Johnson & Son, Inc.  
Parafilm is a registered trademark of Bemis Company, Inc.  
ThermalSeal is a registered trademark of Excel Scientific, Inc.

JC,MD,KH,MAM 08/17-1