ADMEcell

CACOREADY™

Tips for Success

UPON RECEIPT OF THE CACOREADY™ KIT

- Remove the plastic bag containing the CacoReady™ plates from the outer and inner shipping boxes, and place the bag in a safe place protected from vibration or jarring.
- Keep the plates protected from light. We recommend placing them in a secondary container.
- The plates should be stored at 20–25 °C

CHANGING THE SHIPPING MEDIUM

- Conditions for liquefying the shipping medium:
  - Temperature: 37 °C.
  - Incubation period: 4 hours.
- Medium must to be warmed to 37 °C before use.
- Be very careful to avoid touching the cell monolayer when handling the CacoReady™ plates.
- While changing the CacoReady™ shipping medium, perform the steps as quickly as possible.
  - We highly recommend using a 24- or 96-well manifold to remove the cell culture medium.
  - We highly recommend using a multichannel pipette to dispense the cell culture medium.
  - Never change the shipping medium on more than ***one*** plate at a time.
- The shipping medium in ***both*** the apical and the basal compartments should be changed
  - Follow the medium-changing steps in the CacoReady™ Instructions for Use, in the order given.
  - Remove the medium from the basal compartment first, followed by the medium in the apical compartments.
  - Then, re-fill the apical compartments first, followed by the basal compartment.
  - While removing or replacing fluids, keep the basal and apical compartments separate.
- Plates should be returned to the incubator (37 °C) immediately after the medium has been replaced.

TEER MEASUREMENT

- Avoid contacting the cell monolayer with the electrode. If the recommended electrode is used for the 96-Transwell HTS plate, there will be no direct contact with the cell monolayer.
- For optimal TEER results, the cell culture medium should not be changed for 48 hours prior to measurement.
- Taking multiple TEER measurements in a short period of time can alter the cell monolayer stability, so we recommend leaving the plate in the incubator for a minimum of 30 minutes after carrying out TEER measurements.

PERMEABILITY ASSAYS

- The transport buffer (1X HBSS – Ca^{2+}/Mg^{2+} buffer and working solutions (Luciferase Yellow solutions, standard solutions for quality control of the barrier system, or test compound solutions) must be warmed at 37 °C for a minimum of 20–30 minutes prior to performing permeability assays.
- We highly recommend using a 24- or 96-well manifold to remove the culture medium and a multichannel pipette to dispense the culture medium.
  - Using the recommended 96-well manifold can avoid drying up the apical compartments in the 96-Transwell HTS plates. Refer to the CacoReady™ Instructions for Use for specific instructions.
  - Increasing the apical volume is not recommended.
  - Be careful to avoid damaging the cell monolayer.
- We highly recommend performing sample recovery from the basal compartments.
- If it is necessary to recover samples from the apical compartments, use great caution to avoid touching the cell monolayer.
- Always keep the basal and apical compartments separate in order to avoid splashing or leaking.
- Assay design should include an incubation period of one hour maximum.