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MedTech BARCELONA by Leitat

24-Well CacoReady User's Manual

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Product Description

CacoReady is an *in vitro* cell-based model built on 21-day seeded human colorectal adenocarcinoma-derived cells (Caco-2), which resemble the intestinal epithelial barrier.

Caco-2 cells are considered a reference cellular model for assessing compound intestinal toxicity and their mechanisms of action. Furthermore, CacoReady can easily be adapted for high-throughput screening in drug discovery.

CacoReady is delivered in a 24-well/plate format with a unique Shipping Medium (a gel-like cell culture medium) established by MEDTECH BARCELONA, which enables the transport of cells at room temperature and in a ready-to-use format.

Intended Use

This product is mainly indicated for *in vitro* evaluation of compounds:

- intestinal toxicity (e.g., cytotoxicity, genotoxicity, apoptosis, oxidative stress)
- mechanisms of action
- inflammatory and anti-inflammatory activities
- others

NOTE: *This cell-based model is intended for scientific research purposes only. Not for human or veterinary use.*

Principle

Caco-2 cells are an immortalized cell line derived from a human colorectal adenocarcinoma. These cells spontaneously differentiate into a monolayer that exhibits specific properties of absorptive intestinal epithelial cells, such as a well-defined brush border and intercellular junctions.

This cell line seeded on 24-well plastic plates is widely used to screen for intestinal adverse drug effects. Although limitations such as their tumor origin and the absence of the intestinal physiological context must be considered when extrapolating the data obtained *in vitro*, this cell line has the advantage of being easy to handle, reproducible, and adaptable to automatic high-throughput screening, which makes them relevant as an excellent toxicokinetic tool for predicting intestinal adverse drug effects and mechanism-based toxicity.

In the experimental setup, these cells are seeded for 21 days on 24-well plates (CORNING Cat# 3524) to form a tight cell monolayer.

Handling and experimental procedures are provided below. The manual has been written for users with experience in cell culturing. For more detailed advice, please contact us at:

reagents@medtechbcn.com

Timeline for Delivery and Experimental Procedures

- Day 1: Start of Production (Seeding of cells)
- Days 14-15: Package Dispatch (depending on destination)
- Days 16-17: Package Delivery
- Day 18: Replacement of Shipping Medium (liquefaction)
- Day 21-25: Medium Replacement/Assay Performance (see Table 1)

Packages are dispatched on Mondays/Tuesdays and delivered within 24-48 h to EU countries, 48-72 h to USA, and 48-96 h to Asian countries. For other locations and customized schedules, please contact us at:

reagents@medtechbcn.com

The recommended timing overview for compound testing is Day 21 (Monday) (see Figure 1 for details).

CacoReady	Monday	Tuesday	Wednesday	Thursday	Friday
Week 0					**12:00 p.m. (CET) last ordering day**
Week 1	*Pre-Production*	*Start of Production Day 1*			
Week 2	*Day 7*				
Week 3	*Shipment Day 14*	Reception of Plates			*Liquefaction Day 18*
Week 4	*Day 21*	Perform Assay			*Day 25*

Figure 1. Timeline of manufacturing and operation for CacoReady in 24-well plastic plates.

In case you choose to conduct the experiments later, the medium replacement must be carried out as follows:

DAY OF EXPERIMENT	MEDIUM REPLACEMENT
Tuesday (Day 22)	Monday (Day 21)
Wednesday (Day 23)	Monday (Day 21)
Thursday (Day 24)	Monday (Day 21) Wednesday (Day 23)
Friday (25)	Monday (Day 21) Wednesday (Day 23)

Table 1. Recommended day for medium replacement.

NOTE: *These steps enable the planning of the assay according to the user´s convenience.*

Equipment (not included)

- Cell culture laminar flow hood
- CO2 incubator
- Water bath
- Multichannel pipettes
- Automatic multichannel micropipette (recommended)
- Aspiration system
- **24-well format vacuum manifold (Drummond Cat# 3-000-097 recommended)**

Consumables

- Sterile culture medium containers (i.e., Costar 50 ml, Cat# 4870) *(not provided)*
- 15 and 50 mL conical tubes and 1.5 mL Eppendorf tubes *(not provided)*
- Pipette tips *(not provided)*

Solutions (may be included)

NOTE: *MedTech Barcelona can supply Cell Culture Medium if required.*

- **Caco-2 Cell Culture Medium**: Dulbecco's Modified Eagle's Medium - low glucose (1 g/L) (SIGMA Cat# D5546) supplemented with (final concentrations):
  - 10 % V/V Fetal Bovine Serum (BIOWEST Cat# DE14-801F)
  - 2 mM L-glutamine (LONZA Cat# BE17-605F)
  - 100 U/mL; 0.1 mg/mL Penicillin-Streptomycin (LONZA Cat# DE17-602F)

NOTE: *If the specified reagents are not available, other reagents with similar features and specifications can be used.*

Handling

Upon reception, retrieve the zipped bags containing the plates. Open the zip and leave the bag in a dark location at room temperature until Day 18 (refer to Timeline; Figure 1).

Replacement of Shipping Medium

CAUTION: *Never handle more than one plate at a time while changing the shipping medium. Re-solidification of the shipping medium may damage the cell monolayer.*

These **steps** will be **carried out on Day 18** (refer to Timeline; Figure 1). Perform all manipulation under sterile conditions.

1. Retrieve the plates from the bags and remove the parafilm wrap.
2. **Incubate** the plates in a 5 % CO2 humidified atmosphere at 37 °C for **2 hours** until the **shipping medium** reaches **liquefaction**.
3. Remove one CacoReady plate from the incubator and place it inside the laminar flow hood.
4. Using sterile procedures (**inside the laminar flow hood**), fill a sterile reagent reservoir with 20 mL of pre-warmed (37 °C) Caco-2 cell culture medium.
5. Open the CacoReady plate and leave the lid upwards, next to the plate.
6. Using the aspiration manifold connected to a vacuum pump (adjust aspiration flux to medium-low), aspirate the liquefied shipping medium, taking care not to disrupt the monolayer. Make sure the shipping medium has been removed from all wells. A remanent medium will be left in each well.
7. Using a multichannel pipette, dispense **500 µL** of Caco-2 cell culture medium from the sterile reservoir, and fill the **24-wells** of the CacoReady plate, column by column. Always add the medium against the wall of the well and not directly onto the cell monolayers.
8. Replace the lid and place the plate inside the cell culture incubator, set at 37 °C and 5 % CO2.
9. Once the shipping medium has been substituted by fresh Caco-2 cell culture medium, the plates should be placed inside the incubator until next Monday (Day 21). **Replacement with a new fresh medium** will be carried out once (Day 21) or twice (Days 21 and 23), depending on the day of the assay (refer to Table 1 for details), following the procedure described above.

Assay Performance

Perform the assay as needed. Please, feel free to contact us with any queries or doubts.

Quality Controls

This section provides the morphology of 21-day differentiated Caco-2 cells seeded on plastic dishes and the pre-specified criteria of monolayer confluency for batch release and after recovery from the shipping medium (SM).

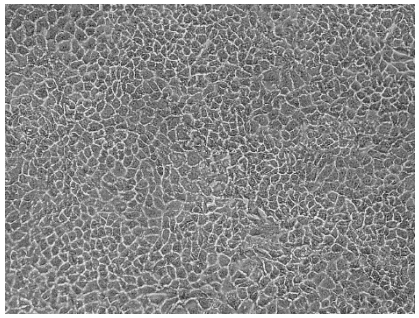
	Monolayer confluency	
Cell morphology	Batch release	Recovery from SM
	≥ 95 %	≥ 90 %

Figure 2. morphology of 21-day differentiated Caco-2 cells.