



CACOREADY™

## A Novel, Ready-to-Use System for In Vitro Intestinal Absorption Evaluations

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## INTRODUCTION

The CacoReady™ system is a new ready-to-use concept for *in vitro* intestinal absorption evaluation. It is an integrated tool designed to minimize time and costs for preclinical screening of oral compounds. CacoReady™ is perfectly suited for assessing permeability and can easily be adopted for high-throughput screening of compounds.

CacoReady™ is an innovative cell-based assay with the following features:

- Provides differentiated Caco-2 barrier (21-day system), plated on HTS Transwell® -24 plates or HTS Transwell® -96 plates.
- Transwells are integrated to allow easier handling.
- Patented, proprietary shipping medium is stable at room temperature allows up to 5 days of transportation/storage at room temperature.
- User-friendly system and easy handling.
- Cost-effective for any size laboratory.

The CacoReady™ system is designed in collaboration with industrial partners and validated independently by several biotech and pharmaceutical companies

## KEY FEATURES AND BENEFITS

Features	Benefits
Ready-to-use kits are stable at room temperature	Easy handling and storage
Allows end users to avoid in-house maintenance and handling of Caco-2 cell culture	Reduces operative costs and provides significant time savings
User-friendly and suitable for automation	Adaptable to high throughput automated systems
Cost savings both in direct and indirect costs	Avoids reagent preparation, plastic ware and many hours of tedious cell culture work
	No requirement for cell culture facility, equipment, logistics
Flexibility of the CacoReady™ system	Allows testing of poorly water-soluble components
The exclusive and proprietary shipping media	Ensures the stability of the cell barrier properties
CacoReady™ kits can be used up to 9 days after delivery	Provides flexibility in designing and planning experimental work

TABLE 1. Features and benefits of CacoReady™ system.

## TECHNICAL DESCRIPTION

### Kit Contents

- HTS Transwell® -24 or -96 plates
- Technical sheet (brief description of the kit, quality control, instructions for use)

### Applications

CacoReady™ is intended for *in vitro* evaluation of:

- Oral absorption efficiency
- Compound screening
- Oral bioavailability
- Oral toxicity
- Mechanisms for oral/intestinal absorption
- Research on new delivery systems

### Assay Types

CacoReady™ can be used to perform the following assays:

- Permeability assays (for recommended volumes please consult the product User Manual)
- Immunocytochemistry
- Protein extraction

### Description

The CacoReady™ kit consists of differentiated Caco-2 cell barriers, shipped on day 13 of differentiation, and provides 14-day polarized cultures of Caco-2 cells on polycarbonate microporous filters in 24- or 96-well plates (Figure 1).

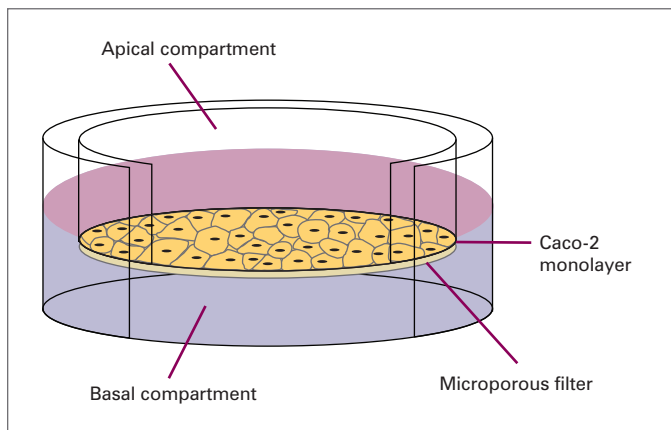


FIGURE 1: Representation of polarized Caco-2 monolayer in Transwell® system.

The innovative CacoReady™ shipping medium maintains the properties and integrity of the cell barrier, providing users with a robust screening tool that can be used up to 9 days after its been received. Figure 2 shows confocal analysis of occludin staining revealing tight junctions status in the CacoReady™ cell barrier.

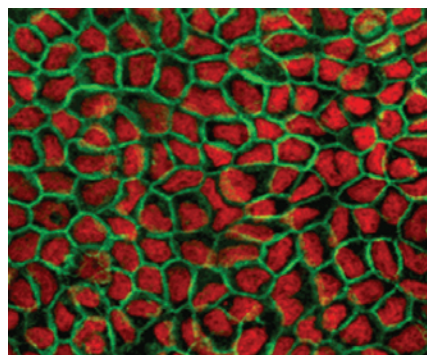


FIGURE 2: Confocal analysis of occludin staining (green) revealing tight junctions status in the CacoReady™ cell barrier.

Figure 3 shows the apparent permeability coefficient of standard compounds characterization.

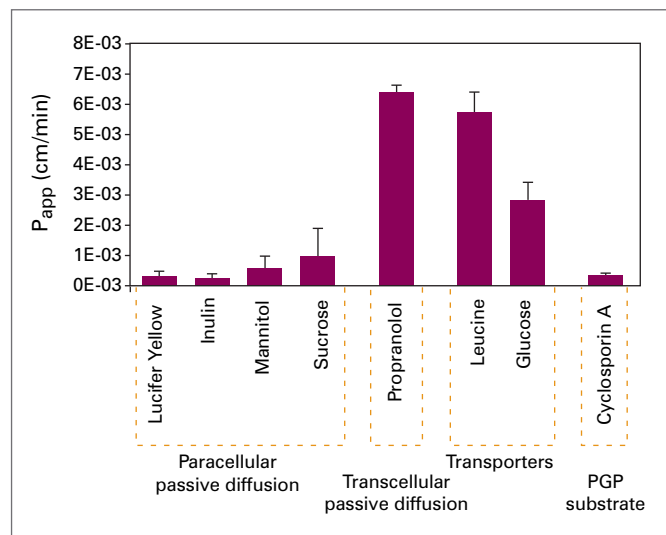


FIGURE 3: Apparent permeability coefficient of standard compounds characterization.

## BIOSAFETY

*For research use only*

This product contains cell lines from human origin and as with any material produced from human origin, it is recommended to observe full safety precautions when handling this material.

The following information is provided with each lot of products:

- Lot Number`
- Transepithelial Electrical Resistance (Ohm.cm<sup>2</sup>)
- Lucifer Yellow Paracellular Flux (%)
- Apparent Permeability (cm/min)
- Manufacturing Date
- Expiration Date

## SHIPPING, HANDLING & UNPACKING THE CACOREADY™ KIT

Note: This is a general overview for handling, unpacking and further processing of CacoReady™ products. For more detailed and comprehensive instructions please refer to the product User Manual.

### Shipping

CacoReady™ is shipped Mondays through Thursdays from the production facility in California. Normal delivery time is 1 or 2 days within the continental United States, Hawaii, Canada and other US territories. Therefore, you should receive the kits between Tuesday and Friday of the week they are shipped. You do not need to work with the plates until Friday.

### Unpacking

- 1 Upon receipt, open the outer box and remove the inner box.
- 2 Open the inner box and remove the plastic zip lock bags containing the HTS Transwell® -24 plates. Leave the zip lock bags open.

The plastic zip lock bags containing the HTS Transwell® -24 plates should be kept at room temperature (15–25 °C) until Friday of the week they are received.

Note: the shipping medium must be changed on Friday of the week the kit is received.

### Changing the Shipping Medium

- 1 On Friday, open the plastic zip lock bags and remove the HTS Transwell®-24 plates (still at room temperature).
- 2 Unwrap the HTS Transwell®-24 plates and carefully pull off the Parafilm.

- 3 Place the HTS Transwell®-24 plates in the cell culture incubator for 4 hours to allow the transport medium to completely liquefy.
- 4 While the shipping medium is liquefying, prepare everything you will need to replace the shipping medium with fresh Caco-2 cell culture medium:
  - Cell culture biosafety cabinet.
  - Standard Caco-2 cell culture medium, pre-warmed to 37°C.
  - Aspiration system.
  - Automatic multichannel micropipettor.
  - Standard 24-well plates, Costar 3526 (for as many as CacoReady™ plates as you have received).
  - Sterile containers for culture medium.
- 5 Prepare the basal plates for changing the medium:
  - a Fill a sterile container with 50 ml of Caco-2 medium that has been warmed to 37°C.
  - b In the biosafety cabinet, unwrap one 24-well basal plate (Costar 3526) for each CacoReady™ plate. Open the plates and place the lids by the plates, facing upwards.
  - c Program the automatic multichannel pipette for a 900 µl sequence. Aspirate 900 µl of warm (37°C) Caco-2 medium from the sterile container, and dispense 900 µl of this medium into each of the 24 wells of the basal plates.
  - d Put the lid on each plate, and place the plates in the incubator.
  - e Re-warm the Caco-2 medium by placing it back in the 37°C bath.

Note: Culture media should be replaced every 48-72 hours.

Once the shipping medium has been liquefied, replace it with fresh Caco-2 culture medium following the steps below. Steps 2–10 must be performed using sterile technique in the biosafety cabinet.

**Caution:** Never handle more than one plate at a time while changing the shipping medium. Re-solidification of the shipping medium could provoke mechanical damage to the cell monolayers.

- 1 Take one CacoReady™ plate and one basal plate out of the incubator and place them both in the biosafety cabinet, oriented the same direction.

- 2 Fill a sterile container with 50 ml of pre-warmed (37°C) Caco-2 culture medium.
- 3 Open the CacoReady™ plate and the basal plate, placing the lids next to the plates, facing upwards.
- 4 Gently lift up the apical (upper) section of the CacoReady™ plate and place it on the basal plate.
- 5 Remove all the liquefied shipping medium from the basal compartments of the CacoReady™ plate by aspirating with the manifold or a Pasteur pipette.
- 6 Gently move the apical section of the CacoReady™ plate from the basal plate back to the top of the original CacoReady™ plate.
- 7 Remove all the liquefied shipping medium from the apical compartments of the CacoReady™ plate by aspirating with the manifold or a Pasteur pipette.
- 8 Cover the CacoReady™ plate with its lid, then put back into the cell culture incubator.
- 9 Repeat steps 1–9 for each plate.

After the shipping medium has been changed to fresh Caco-2 medium, the plates should be kept in the incubator until Monday.

### RESISTANCE OF THE CACOREADY™ SYSTEM TO COMMON SOLUBILIZERS (SOLVENTS)

Absorption of orally administered drugs is mainly determined by their membrane permeability and solubility in the intestinal lumen. As many compounds entering development have high lipophilicity, solubilizers are often used to enhance the bioavailability of drugs having poor aqueous solubility. Accordingly, the potential of solvents to disrupt the barrier status is one of the largest factors limiting wider use of the Caco-2 system. The study presented below focuses on the effect of different solubilizers on the barrier status of the CacoReady™ system, performed to determine the predictability of the model for the oral absorption of poorly water-soluble drugs in humans.)

Lucifer yellow paracellular permeability values obtained in presence of various solubilizing excipients and solvents such as polyethylenglycol (Solvent 1, up to 5%), propyleneglycol (Solvent 2, up to 10%), dimethyl sulfoxide, DMSO (Solvent 3), acetonitrile (Solvent 4) and methanol (Solvent 5, up to 2%; recommended: 0.5%)

were compared to those obtained using only a HBSS control (Figure 4). The permeability values show that the CacoReady™ 21-day barrier remains stable after exposure to the solvents. The results support the use of the CacoReady™ system for poorly water-soluble drugs, to predict their intestinal absorption in humans.

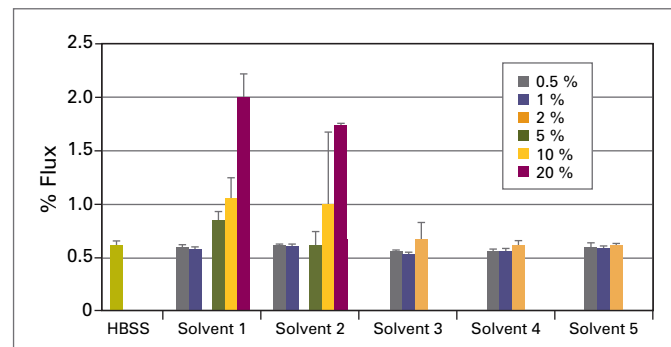


FIGURE 4: Lucifer yellow paracellular flux through the CacoReady™ barrier in the presence of different solvents (after 1 hour solvent exposure).

### STABILITY OF CACOREADY™ DURING SHIPMENT & STORAGE

#### Stability of CacoReady™ During Shipment

The functional stability of CacoReady™ as a ready-to-use product during shipping and storage is presented below.

Thirteen-day Caco-2 cell monolayers were maintained in shipping medium for 1, 3, 4, 5, and 7 days, and barrier status was evaluated by TEER measurement before applying and 72 hours after removing the shipping medium. The CacoReady™ barrier status showed no reduction in barrier properties until 5 days in shipping medium. This indicates that CacoReady™ can be stored and transported at room temperature up to 4 days without loss of Caco-2 barrier properties.

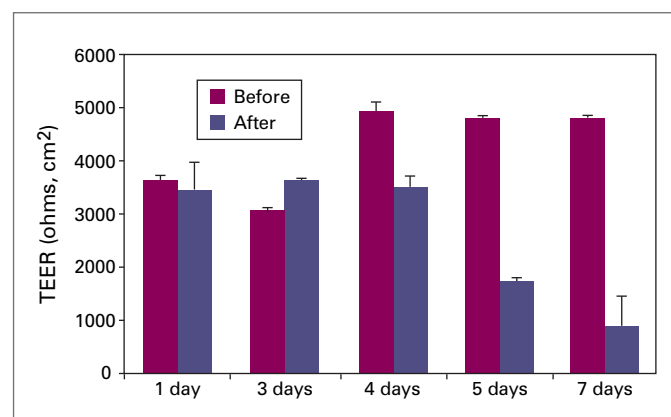


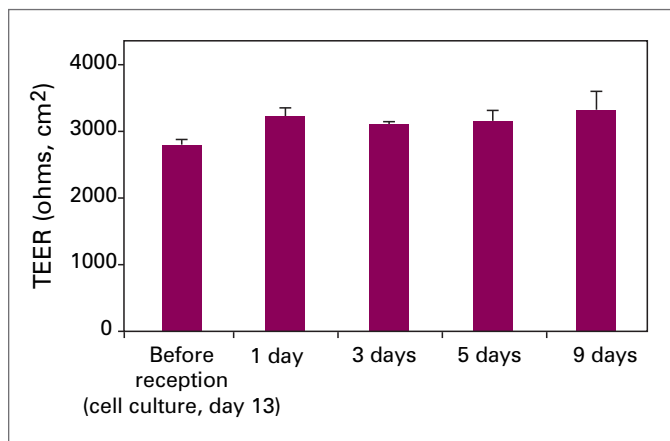
FIGURE 5: Stability of CacoReady™ in shipping medium during shipment and storage.

### Stability of CacoReady™ After Receipt

One of the greatest benefits of the CacoReady™ System is its “ready-to-use” and “user-friendly” aspect. The stability of the kit (i.e., how long after delivery the plates can be stored before barrier properties are lost) is presented below.

Immobilization was maintained for 72 hours at room temperature. The shipping medium was removed and TEER (Trans Epithelial Electrical Resistance) was measured after 1, 3, 5, and 9 days in standard culture conditions.

No changes were observed in the TEER values throughout 72 hours of immobilization. The stability of the CacoReady™ barrier from 1 to 9 days after immobilization is displayed below (Figure 6). This data demonstrates the flexibility the CacoReady™ system brings to designing and planning experiments.



**FIGURE 6:** Stability of the Caco-2 barrier evaluated by TEER measurement at different days after immobilization in CacoReady™ shipping medium.

### REFERENCES

- 1 Le Ferrec et al. “*In vitro* Models of the Intestinal Barrier”. 2001 ATLA 29, 649-668. *ECVAM Workshop Report 46*.
- 2 Yee S. “*In vitro* permeability across Caco-2 cells (colonic) can predict *in vivo* (small intestinal) absorption in man—fact or myth”. *Pharm Res.* 1997 Jun; 14(6):763-6.
- 3 Takahashi Y, Kondo H, Yasuda T, Watanabe T, Kobayashi S, Yokohama S. “Common solubilizers to estimate the Caco-2 transport of poorly water-soluble drugs”. *Int J Pharm.* 2002 Oct 10; 246(1–2):85-94.
- 4 Artursson P, Karlsson J. “Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells”. *Biochem Biophys Res Commun.* 1991 Mar 29;175(3):880–5.
- 5 Rubas W, et al. “Flux measurements across Caco-2 monolayers may predict transport in human large intestinal tissue”. *J Pharm Sci.* 1996 Feb; 85(2):165–9.
- 6 Stewart BH, et al. “Comparison of intestinal permeabilities determined in multiple *in vitro* and *in situ* models: relationship to absorption in humans”. *Pharm Res.* 1995 May; 12(5):693–9.

## OTHER CACOREADY™ PRODUCTS

### **CacoGoblet**

Mucus-secreting CacoGoblet represents a predictive model for compounds or formulations with a passive diffusion transport pathway. Similar to CacoReady™, this product is also designed as a ready-to use kit and has been designed to eliminate in-house cell culture work. CacoGoblet is offered in 24- and 96-well plates.

### **TransportPlus**

This ready-to-use kit provides polarized cultures of Caco-2 and/or TC7 cell barriers and/or MDCK, and is designed to allow comparative permeability evaluation in a single assay with 2 or 3 different cell lines. As with CacoReady™ and CacoGoblet, it is designed to eliminate in-house cell culture work, enabling researchers to allocate more of their resources to focus on their core research.

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