xCELLigence Real-Time Cell Analysis

Co-culture Device Protocol

1. Introduction

Cell–cell interactions are key to biological processes. The new Insert enables investigation of spe-cell-cell interactions in real time, maintaining the cells in separate partments.

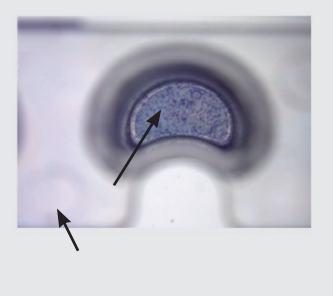


Figure 1. Stained H295R cells on the E-Plate Insert 0.4 µm pore size membrane, showing one E-Plate Insert well and its access port.

Two different cell populations are separated by a 0.4 µm pore size membrane, allowing control of both physical contact and the duration of interactions.

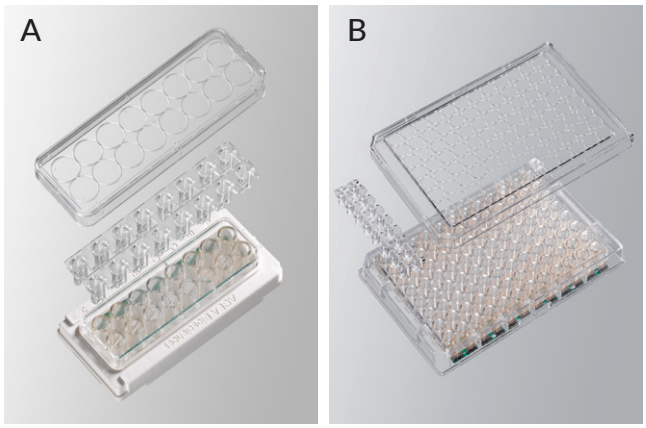


Figure 2. The same 16-well E-Plate Insert is used for both 16- and 96-well formats of the E-Plate and E-Plate VIEW.
A: E-Plate Insert in combination with the E-Plate 16.
B: E-Plate Insert in combination with the E-Plate 96; up to six E-Plate Inserts can be used with each E-Plate 96.

The E-Plate Insert is a 16-well strip compatible with both 16- and 96-well E-Plate formats. Well characteristics (size, shape, spacing, volume) of both E-Plate formats are similar.

- **Easily add compounds or replace media during an experiment:** The E-Plate Insert access port enables access to the lower E-Plate well after assembly (Figure 1).
- **Perform real-time co-culture experiments under physiological conditions:** E-Plate Inserts enable monitoring of indirect cell-cell interactions in standard CO_2 incubators.
- **Use the same E-Plate Insert with multiple E-Plate formats:** The 16-well E-Plate Insert strips fit the E-Plate 16, E-Plate 96, E-Plate VIEW 16, and E-Plate VIEW 96 (Figure 2) for use with xCELLigence RTCA DP, SP, MP, and Cardio Instruments.

2. Materials and Devices

Product	Catalog Number	Pack Size
E-Plate Insert 16	06465382001	6 Inserts, 6 Receiver Plates* 16
E-Plate Insert 96	06465412001	36 Inserts, 6 Receiver Plates 96
E-Plate Insert 96 Accessories	06465455001	6 Receiver Plates 96

3. Protocol:

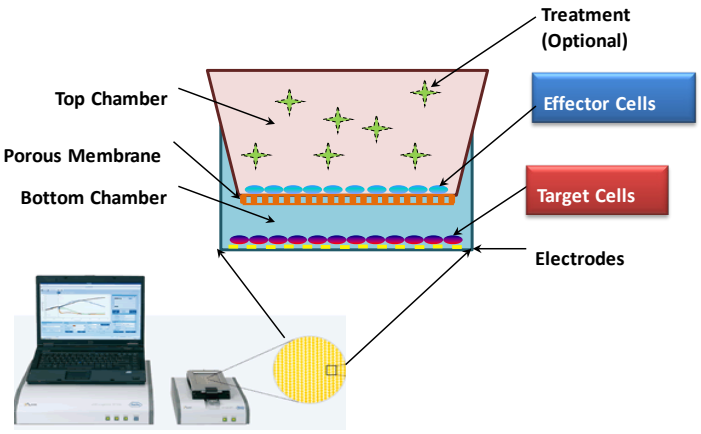
Day 1:

For E-Plate 16/96:

- Add 50 µL of assay media to the wells of the E-Plate 16/96 and return to station.
- Take the background measurement.
- Remove the E-Plate 16/96 from the station and return to the tissue culture hood.
- Suspend the target cells at the appropriate concentration in 50 µL and add this volume to each well. → 100 µL final in the E-Plate 16/96 wells (including background media).
    - Target cells = cells responding to stimuli and whose impedance will be measured.

For E-Plate Insert:

- Suspend the effector cells in 60 µL of assay media and add this volume to each well in the E-Plate Insert.
  - Effector cells = cells stimulating the factor/molecule/stimuli. Impedance of these cells will not be measured as they will be cultured in the E-Plate Insert device.
- Add 120 µL of assay media below each of the E-Plate Insert wells for overnight incubation.
  - This 120 µL of assay media is added below the insert in the E-Plate Insert Receiver Plate. This ensures that the membrane equilibrates with the media overnight from both sides.



  - Note: It is recommended that the E-Plate Inserts and E-Plate 16/96 devices are incubated independently/separately overnight prior to assembly for the assay. This ensures that the cells settle and attach evenly in both devices before the two are combined.
- Incubate both the E-Plate 16/96 and the E-Plate Insert (in the receiver plate) in the tissue culture hood for 30 minutes.
  - This allows for uniform seeding of the cells on both the E-Plate 16/96 as well as on the E-Plate Insert membrane.
- Incubate and monitor the E-Plate 16/96 device overnight as the target cells adhere and proliferate.
- Incubate the E-Plate Insert (in the receiver plate) in the same incubator overnight.

Day 2:

- Remove the separate E-Plate 16/96 device (from the station) and E-Plate Insert (from the incubator)
- Remove the E-Plate Insert from the receiver plate, and carefully add the entire device directly to the wells in the E-Plate 16/96.
  - Add media back (from the receiver plate) into the E-Plate Insert wells if you notice a considerable amount of media getting "pulled through" upon removal from the receiver plate.

Continue monitoring for 3-5 days.

4. Sample Data

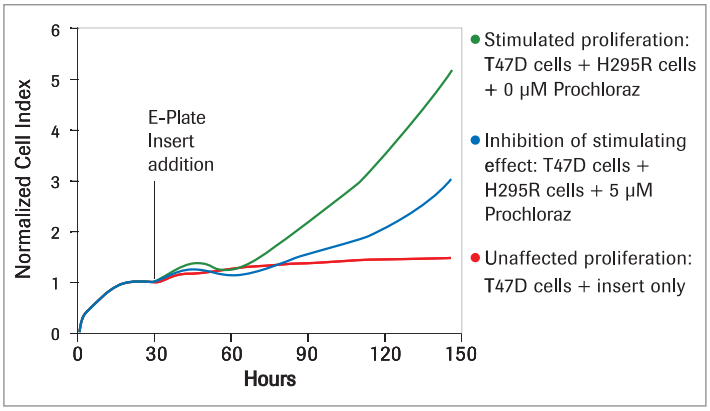


Figure 3. Real-time monitoring of co-culture-induced proliferation stimulation and its inhibition using the E-Plate Insert. Intercellular interactions play an important role in normal cell development and tumorigenesis. Results show that the proliferation of hormone-responsive tumor cells is likely mediated by hormones and growth factors exchanged between the two cell populations separated by the E-Plate Insert.
Elevated T47D cell proliferation on the E-Plate (green trace ■) was induced by hormone secretion of H295R cells in the insert, and inhibited by the hormone synthesis inhibitor Prochloraz (blue trace ■). Incubation of T47D cells with only the E-Plate Insert did not affect proliferation (red trace ■).