User Guide

Agilent
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xCELLigence Real-Time Cell Analysis

Coculture device instructions

Introduction

Cell-cell interactions are key to understanding biological processes. The Agilent E-Plate Insert enables investigation of specific cell-cell interactions in real time, while maintaining the cells in separate compartments.

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

Well characteristics (size, shape, spacing, volume) of both E-Plate formats are similar. The main advantages of using the inserts are:

- Easily adding compounds or replacing media during an experiment. The E-Plate Insert access port enables access to the lower E-Plate well after assembly (Figure 1).
- Performing real-time coculture experiments under physiological conditions. E-Plate Inserts enable monitoring of indirect cell-cell interaction in a standard CO_2 incubator.
- Using the same E-Plate Insert with multiple E-Plate formats. The 16-well E-Plate Insert strips fit the Agilent E-Plate 16, E-Plate 96, E-Plate VIEW 16, and E-Plate VIEW 96 (Figure 2) for use with Agilent xCELLigence RTCA DP, SP, MP, and Cardio instruments.

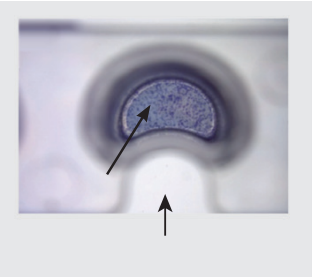

Figure 1. Stained H295R cells on an Agilent E-Plate Insert 0.4 µm pore size membrane, showing one E-Plate Insert well and its access port.

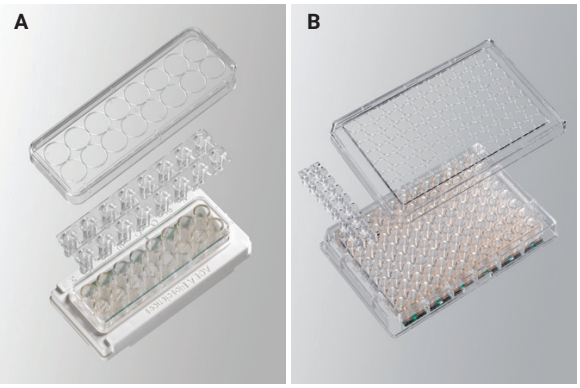

Figure 2. The same 16-well E-Plate Insert is used for both 16- and 96-well formats of the Agilent E-Plate and E-Plate VIEW. (A) The E-Plate Insert with the E-Plate 16. (B) The E-Plate Insert in combination with the Agilent E-Plate 96; up to six E-Plate Inserts can be used with each E-Plate 96.

Materials and devices

Product	Part Number	Contents
E-Plate Insert 16	6465382001	6 Inserts, 6 receiver plates* 16
E-Plate Insert 96	6465412001	36 Inserts, 6 receiver plates 96
E-Plate Insert 96 accessories	6465455001	6 receiver plates 96

Instructions

Day 1

For the E-Plate 16/96:

- Add 50 µL of assay media to the wells of the E-Plate 16/96 and return to the 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 (cells that respond to the stimuli, and whose impedance will be measured) at the appropriate concentration. Add 50 µL to each well. The final volume in the E-Plate 16/96 wells (including background media) should be 100 µL.

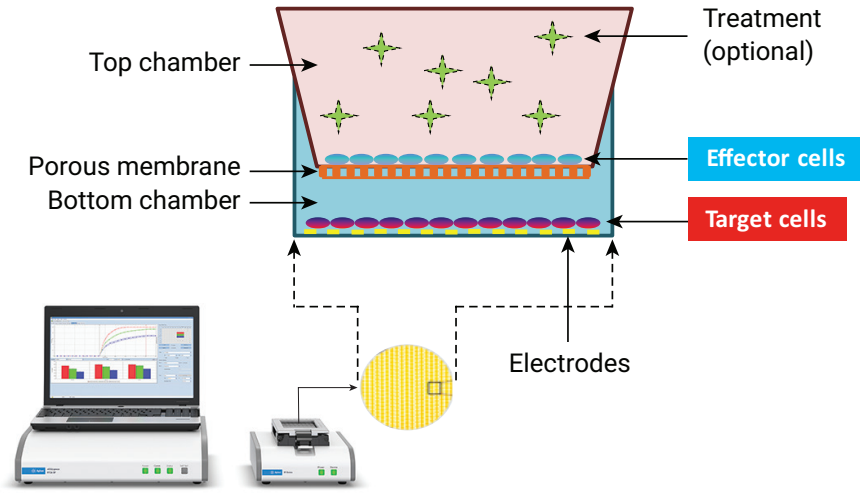

Figure 3. Agilent E-Plate layout.

For the E-Plate Insert:

- Add 120 µL of assay media to each well of the receiver plate for overnight incubation.
    - The 120 µL of assay media added to the lower receiver plate ensures that both sides of the membrane equilibrate with the media.
- Suspend the effector cells (cells producing the factor/molecule/stimuli; the impedance of these cells will not be measured, as they will be cultured in the E-Plate Insert device) in the assay media, and add 60 µL to each well of the E-Plate Insert.
    - **Note:** It is recommended that the E-Plate Inserts and E-Plate 16/96 devices are incubated separately overnight before assembly for the assay. This ensures that cells settle and attach evenly in both devices before the two are combined.
- Leave both the E-Plate 16/96 and E-Plate Insert (in the receiver plate) in the tissue culture hood for 30 minutes. This allows for uniform seeding of cells on both the E-Plate 16/96 and on the E-Plate Insert membrane.
- Incubate and monitor the E-Plate 16/96 device overnight, using an xCELLigence device 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 E-Plate 16/96 device from the station and E-Plate receiver plate with the insert from the incubator.
- Remove the E-Plate Insert from the receiver plate, and carefully add it directly to the wells of 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” during removal from the receiver plate.
- Continue monitoring for 3 to 5 days.

Sample data

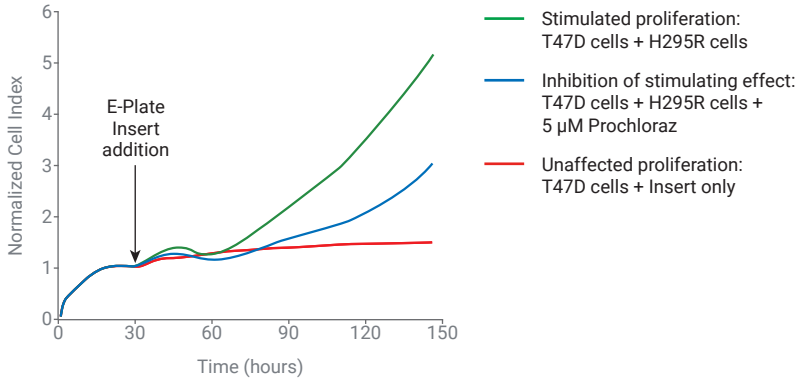

Figure 4. Real-time monitoring of coculture-induced proliferation stimulation and its inhibition using the Agilent E-Plate Insert. Intercellular interactions play an important role in normal cell development and tumorigenesis. Results show that the proliferation of hormone-responsive tumor cells is likely 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).

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