Flexcell® Transwell Holder

The Flexcell® Transwell® Holder supports and raises a Transwell® device so that it rests above the underlying cell monolayer grown on the rubber membrane bottom of a Flexcell® BioFlex® culture plate (Fig. 1-2). The Transwell® Holder allows users to perform co-culture and cell migration assays within a Flexcell® BioFlex® culture plate, thus allowing users to test the effects of secretagogues from strained cells on unstrained cells in the Transwell®. The Transwells® can also act as an unstrained control in the same well as a strained sample.

Transwell® Holders for 6-well Flexcell® BioFlex® plates fit 6-well Transwell® inserts (24 mm insert diameter or 37-41 mm outer lip diameter and 15.5-16.5 mm height), and Transwell® Holders for 24-well Flexcell® HT BioFlex® plates fit 24-well Transwell® inserts (6.5 mm insert diameter or 16.9-18.8 mm lip diameter and 15.5-16.5 mm height). Transwells® should be used according to the manufacturer’s recommended protocol. For more information about Transwell® Holders see the product web page at http://www.flexcellint.com/TranswellHolder.htm.

SAMPLE MIGRATION ASSAY PROTOCOL
1. In a suspension flask, add 1.5 x 10^6 HL60 cells to 10 ml of IMEM (Iscove’s Modified Essential Medium) with 20% FBS and 1.3% DMSO.
2. Incubate 5-7 days at 37°C, 5% CO2 to make cells migration competent.
3. Add 100 µl of prepared hydrogel (i.e., Collagel® or Thermacol®) to the upper chamber of a 6.5 mm diameter Transwell®. Ensure hydrogel spreads evenly and allow to gel.
4. Centrifuge activated HL60 cells and resuspend in IMEM. Dilute to a density of 1.5 x 10^6 cells/ml.
5. Add 100 µl of HL60 suspension to upper chamber.
6. Add chemoattractant of interest to lower chamber.
7. Incubate at 37°C, 5% CO2, 100% humidity for 2 hours.
8. Remove supernatant from Transwell® and gently rinse with PBS.
9. Add cell detection reagent (e.g., Alamar blue, calcein, etc.) and quantify HL60 cells that have migrated into hydrogel using the protocol appropriate for the detection reagent selected.

ORDERING INFORMATION
Transwell® Holders for both 6-well (Cat. No. TW6-3000) and 24-well (Cat. No. TW24-3000) plates are sold in a set of four. They can be reused if they are sterilized by ETO treatment between uses. DO NOT AUTOCLAVE.

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