## 1. Objective

This protocol describes an assay that studies antibody transcytosis across a blood-brain barrier on-a-chip in the MIMETAS OrganoPlate® 3-lane (as published by Wevers et al., DOI: 10.1186/S12987-018-0108-3).

## 2. Background

Therapeutic antibodies can be targeted to the brain to treat brain diseases. In this assay, a combination of a target antibody (which is supposed to cross the BBB) and a control antibody (which is not supposed to cross the BBB) is perfused through the apical compartment of the BBB model (see figure 1). After incubation, samples are taken from the basal compartment. The antibody contents of these samples can be analyzed using ELISA or a Mesoscale Discovery system.

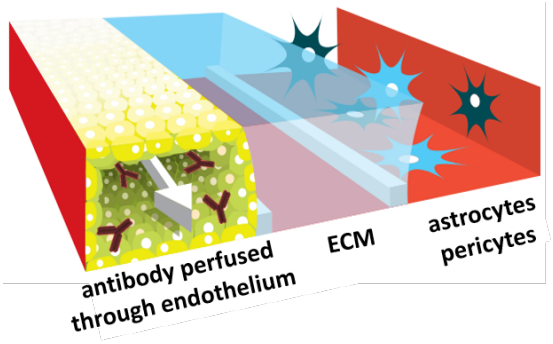



***Figure 1.*** *The OrganoPlate® 3-lane comprises 40 chips, which are used to culture 40 miniaturized BBB models. The BBB model comprises an endothelial microvessel, grown against an extracellular matrix (ECM) gel, and supporting astrocytes and pericytes. Antibody is perfused through the lumen of the endothelial microvessel (the apical compartment). Samples are then taken from the basal compartment and antibody contents are analyzed.*

## 3. Materials

- OrganoPlate® 3-lane with BBB co-cultures (see figure 2)
  - The culture has an endothelial tubule in the first channel (= apical compartment)
  - The culture has astrocytes and pericytes in the third channel (= basal compartment)
- Medium (i.e. ScienCell™ Astrocyte medium, ScienCell #1801)
- Antibodies
  - Target antibody
  - Control antibody
- Multichannel pipette
- Multichannel tips
- OrganoFlow®

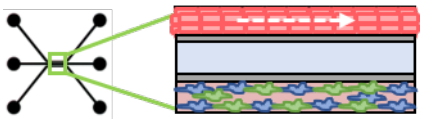


***Figure 2.*** *BBB co-culture of endothelial cells (red), astrocytes (green), and pericytes (blue) in a chip of an OrganoPlate® 3-lane.*

# 4. Assay

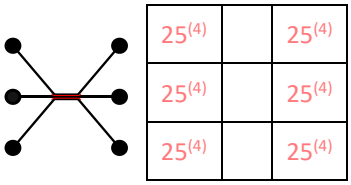
### Prepare antibody solutions

1. Warm medium in a 37°C water bath
2. Prepare the antibody solution in pre-warmed medium at the appropriate concentration
    a. Prepare 70 µL of antibody solution per chip
    b. Optimal antibody concentrations depend on the antibody used
        i. For MEM-189 and anti-HEL antibodies, we used a concentration of 1.25 µM
    c. Ideally, prepare a solution that contains both a target antibody (i.e. from human) and a control antibody (i.e. from mouse). For example, prepare 1.25 µM target antibody + 1.25 µM control antibody in pre-warmed medium. This way, each target has a control measured from the same chip. If the target and control antibody originate from the same species, you can use different chips or adapt the analysis method.

### Wash chips with medium

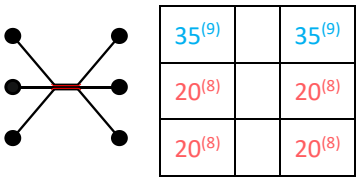
During the culture of the BBB on-a-chip model, only the top channel is perfused with medium. To assure proper flow profiles and correct readouts, it is essential to first perform a "washing step" that wets all the inlets and outlets.

3. Aspirate media from top inlets and outlets
4. Add 25 µL medium to all inlets and outlets (see image on the right)
5. Place plate under an angle and perfuse for 5 minutes
6. Aspirate all media
7. Plate is now ready for starting antibody transcytosis assay




### Antibody transcytosis

8. Add 20 µL of medium without antibody to the middle inlets and outlets and the bottom inlets and outlets of each chip
9. Add 35 µL of antibody solution to the top inlets and outlets




10. Place plate on OrganoFlow® in the incubator for 1 hour
    a. For different endothelial cell types and different antibodies, the incubation time may need optimization
11. Sample from apical and basal compartments by collecting the contents of the associated wells
    a. In case you want to sample from one chip at several time points, you can remove the volume you need from the compartment of interest by pipetting (i.e. 10 µL). You can then decide to not adjust the volumes (resulting in a flow through the system) or to replace the volume taken out with fresh medium (and correct for the change in concentration).

## MIMETAS product list

| Cat. No. | Product Name |
|---|---|
| MI-AR-CC-01 | OrganoReady® Caco-2 |
| 9605-400-B | OrganoPlate® 2-lane |
| 4004-400-B | OrganoPlate® 3-lane 40 |
| 6405-400-B | OrganoPlate® 3-lane 64 |
| 6401-400-B | OrganoPlate® Graft |
| MI-OFPR-S | OrganoFlow® S |
| MI-OFPR-L | OrganoFlow® L |
| MI-OT-1 | OrganoTEER® |

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