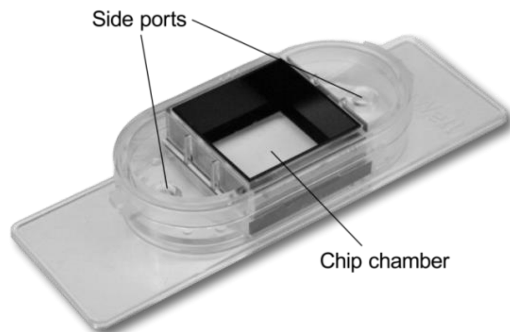
SIEVEWELL™ Slide User Guide

Introduction

Product Overview

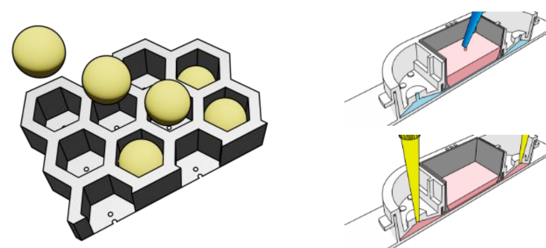


Dimensions (W x D x H)	25 mm x 75 mm x 12 mm
Size of nanowells	20 µm width, 25 µm depth
Number of nanowells	370,000
Chip area	17 mm x 17 mm
Working volume	0.5 mL to 2 mL
Pore diameter	3 µm
Material	Optical grade polymer, PS, PC

- Low attachment surface
- Sterilized by gamma radiation
- DNase free, RNase free, Human DNA free

Principle of Work

Each of 370,000 nanowells of the SIEVEWELL chip has microscopic pores connecting the volume above the chip to two side ports through a micro-gap situated below the chip membrane. The size of the pores allows passing liquids while efficiently retaining cells in the nanowells.



When cells are seeded onto the chip and the buffer is aspirated from side ports, cells are pulled down and individually captured in nanowells following flow lines. A similar manipulation allows to stain and wash cells without cell loss. SIEVEWELL slide can be used for isolation of live or fixed cells. The cells can be stained prior to cell loading or on-chip.

Important points before starting

Do not aspirate too much liquid

Too large aspiration can lead to a partial drying of the gap below the membrane and thus to the air entering into the nanowells. The membrane (bottom of the chamber) should look uniform.

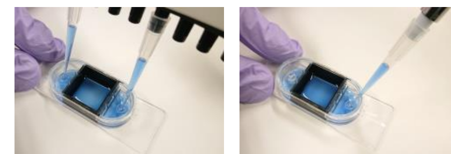
Correct

Correct

Incorrect

Incorrect

Liquid can be discarded from side ports using only P200 pipette with 200 µL tips.
To discard larger liquid volume, one can use a multi-channel pipette and aspirate multiple times,
e.g. to discard 2 mL = 2 tips x 200 µL x 5 times.
If using single channel pipette aspirate by alternating between the two side ports,
e.g. to discard 2 mL = 1 tip x 200 µL x 10 times.



Procedure

To watch a video on how to use the SIEVEWELL Slide, visit **https://www.sievewell.com/procedure**

Equipment and Reagents to be supplied by user

- Single channel pipette P200 or 8-Channel Pipette P200 and tip [P200]
- Single Channel Pipette P1000 and tip [P1000]
- 100% Ethanol
- DPBS

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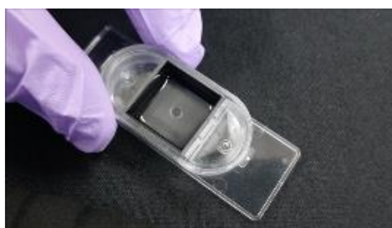
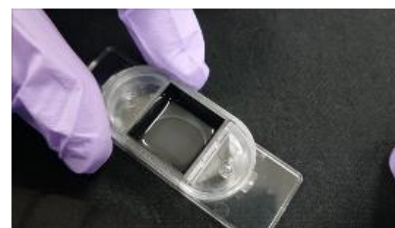
1. Priming with ethanol

Add 0.5 mL of ethanol P1000

Wait 30-60 s until ethanol permeates and spreads below the membrane.





Some ethanol should remain above the membrane as below.

Add 0.5 mL of DPBS.

DPBS progressively permeates through the chip membrane. Some DPBS remains on top of the membrane.

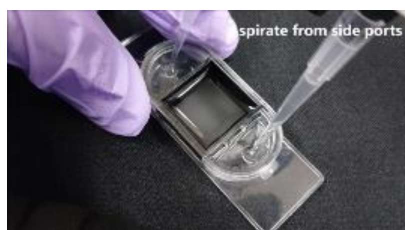
Chamber is filled with PBS.

PBS remains.

2. Wash with DPBS

Add 2 mL of DPBS P1000

Aspirate 2 mL (5 x 2 x 0.2 mL) of DPBS from side ports. P200



Do not aspirate too much, some DPBS should remain above the membrane as below.

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3. Equilibrate with assay medium

This step is to change liquid in nanowell from PBS to required assay medium e.g. RPMI1640 + 10%FBS.

Add 2 mL of assay medium P1000

Aspirate 2 mL (5 x 2 x 0.2mL) of medium from side ports. P200

Repeat again.

Add 2 mL of assay medium.
Aspirate 2 mL of medium.

Do not aspirate too much, some medium should remain above the membrane as below.

Add 0.6 mL of assay medium to avoid drying up of surface. Both upper chamber and the gap below the membrane are filled with assay medium.

4. Cell loading

Prepare cell suspension with 1 mL of assay medium.

Determine the desired number of cells following the table.

Human PBMC loaded	1 cell/well	2 ≥ cells/well
50,000	97.5 %	2.5 %
100,000	95.6 %	4.4 %
200,000	93.3 %	6.7 %

Assay medium should remain above the membrane as below.

Add 1 ml of cell suspension. P1000

Aspirate 1.6 mL (4 x 2 x 0.2 mL) from side ports. P200

Add slowly 1 mL of assay medium.

Now ready for assay.

5. Assay (Immunostaining)

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Standard fixation, permeabilization, blocking procedure may be done before staining. Use required reagent and buffer for required procedure.

Aspirate 2 mL (5 x 2 x 0.2 mL) of assay medium from side ports. P200

Add slowly 1 mL of assay medium.

Aspirate 0.4 mL (1 x 2 x 0.2 mL) from side ports. P200.

Incubate at required temperature for required time.

6. Post-incubation washing

Prepare required washing buffer e.g. DPBS, RPMI1640.

Aspirate 1.6 mL (4 x 2 x 0.2 mL) of assay medium from side ports. P200

Add slowly 1.2 mL of washing buffer.

Aspirate 1.2 mL (3 x 2 x 0.2 mL) from side ports. P200. Repeat washing more 2 times (add 1.2 mL, aspirate 1.2 mL).

Add 1.0 mL of required buffer to avoid drying up of surface. Now ready for imaging. Place the slide directly on microscope.

Safety instruction

When using this product, follow general laboratory precautions and pay attention to safety. Avoid touching the surface of the film with your hand. Read all user manuals thoroughly before using the instrument. Wear appropriate personal protective equipment (PPE) when handling reagents and samples to avoid exposure.

Warranty

SIEVEWELL should be stored dry at room temperature (15 - 25 ºC).

The information in this document is subject to change without notice.

Single use only. Do not disassemble. For research use only.

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