SIEVEWELL Slide User Guide

Please refer to the video manual along with this user guide.

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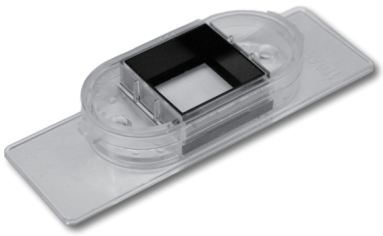


1. General Instruction

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

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

2. Basic Usage

2-1. How to add liquid

1. Hold

Hold both ends of the body tightly during liquid handling.

2. Touch corner

Liquid should be gently flown into the chamber along with the frame wall by touching pipette tip at the corner of the frame.

3. Tilt pipette

Tilt the pipette tip against the device during liquid handling to prevent breaking the membrane. Touching pipette tip to the membrane might break the membrane.

4. Add slowly

Load liquid gently and slowly (*ca.* 5 seconds for 1 mL loading). Gentle liquid loading is important to keep the trapped cells within the nanowells and to obtain a high single cell rate.

2-2A. How to discard liquid [using multi channel pipette ver.]

1. Prepare pipette

Set an 8-channel multi-channel pipette. Attach tips to the 1st and 5th pipette channels.

2. Attach pipette

Tightly put the pipette tips to the holes in two side ports, then aspirate liquid.

Position of side ports in the device is designed to fit with positions of 1st and 5th channel of 8-channel multi-channel pipette. Diameter of the hole in the side port is also designed to fit with the size of a P200 pipette tip.

3. Aspirate slowly

Aspirate proper amounts of liquid slowly with careful attention to level of the liquid surface (*ca.* 3 seconds for 200 µL x 2 ports aspirating).

Do not let the filter membrane dry up. If liquid disappears above the membrane, stop aspirating. **After aspiration, thin layer of liquid remains on the surface of the membrane.** Do not aspirate too much liquid. Once film is dried up, liquid is difficult to penetrate through the membrane and air bubble comes under membrane

About notation of aspirate volume

In this protocol, please follow the notation below when you use a multi-channel pipette.

(e.g.) 2mL, 200 µL x 2 holes x 5 times

2 mL - total amount of aspirating liquid

200 µL - volume setting of multi-channel pipette

2 holes - number of side ports

5 times - count of aspiration when using multi-channel pipette

2-2B. How to discard liquid [using single pipette ver.]

1. Close side ports

Completely close one of side ports with a P200 tip and finger.

2. Attach pipette

Tightly put a pipette tip into a hole in the opposite side port.

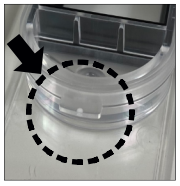
3. Aspirate slowly

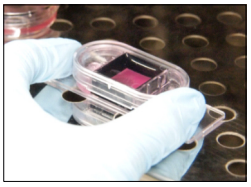
Aspirate slowly and carefully by watching the level of the liquid surface.

Do not let the filter membrane dry up. If liquid disappears above the membrane, stop aspirating. Do not aspirate too much liquid. Once film is dried up, liquid is difficult to penetrate through the membrane and air bubble comes under membrane

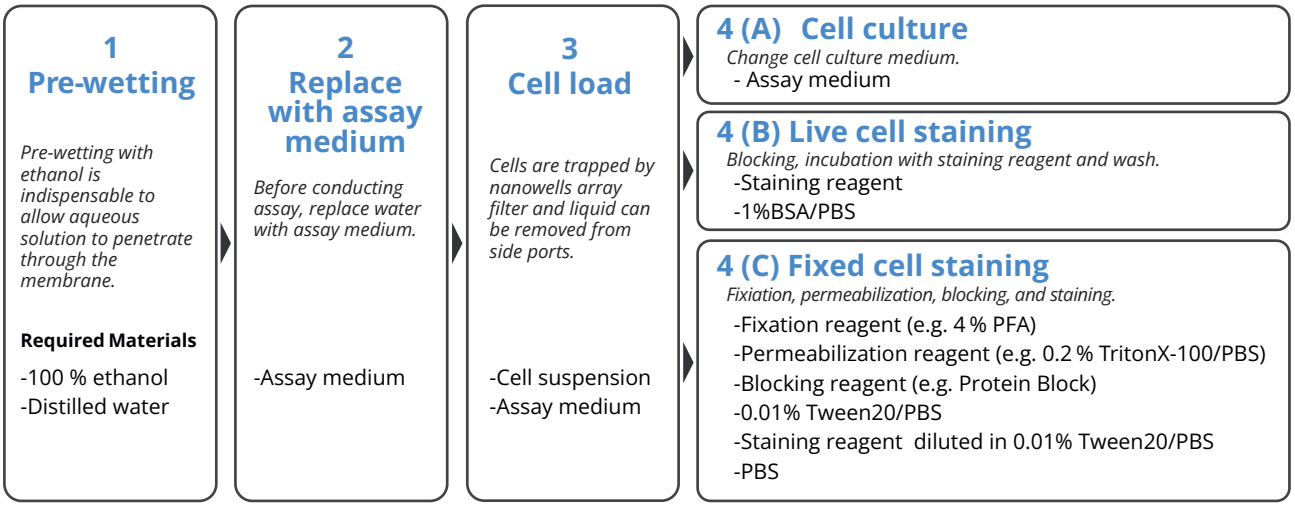
2-3. How to move SIEVEWELL Slide

Please hold the protruding part to move the SIEVEWELL Slide.





3. Workflow Overview, Required Materials



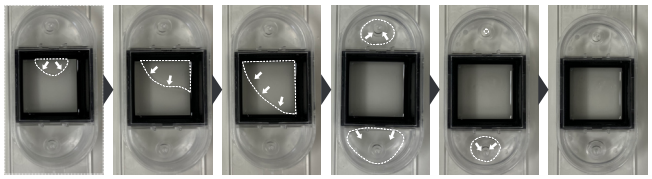
4. Protocol

4-1. Pre-wetting

Add ethanol

1) **Add 1 mL of 100 % ethanol** to the chamber.

2) **Wait until ethanol fully wet the membrane.** Air bubble is generated in about 10 seconds. Check air bubbles reaching to the side ports and disappear completely. (Generally, 30 seconds ~1 minute)



If the wetting of the membrane is not properly done, some air bubbles would remain beneath the membrane or side ports. In this case, wait further minutes until air bubbles disappear.

Replace with distilled water

3) **Immediately add 1 mL** of distilled water to the chamber.

4) **Discard 990 µL** from side ports. **(165 µL x 2 ports x 3 times).**

5) **Add 2 mL** of distilled water to the chamber.

6) **Discard 2 mL** from side ports. **(200 µL x 2 ports x 5 times).**

7) **Repeat** steps 5 and 6 for 2 times.

4-2. Replace assay medium

Examples of assay medium
(A) Cell culture : culture medium (e.g. 10 % FBS/RPMI-1640)
(B) Live cell imaging : 1 % BSA/PBS
(C) Fixed cell imaging: PBS

1) **Add 2 mL of assay medium** to the chamber.

2) **Discard 2mL** from side ports. **(200 µL x 2 ports x 5 times).**

3) **Repeat** steps 1 and 2 for 2 times.

4-3. Cell loading

Recommended number of cells
SWS2001-5 : less than **$2x10^5$** cells /mL
SWS5001-5 : less than **$5x10^4$** cells /mL

1) **Load 1 mL of cell suspension.**

2) **Discard 1 mL** from side ports. **(125 µL x 2 ports x 4 times).**

3) **Go immediately to next step. Do not leave the device more than 5 min at this step.**

4-4. (A) Cell culture

Add medium and incubation

1) **Add 1mL of assay medium** to the chamber.

2) Cover with lid to avoid drying up surface.

3) Move to incubator. Please hold protrude to move.

4) Put SIEVEWELL in incubator as for a cell culture dish.

Replace culture medium

5) **Discard 1 mL** from side ports. **(125 µL x 2 ports x 4 times)**

6) **Add 2 mL of assay medium** to the chamber.

7) **Discard 2 mL** from side ports. **(200 µL x 2 ports x 5 times).**

8) **Repeat** steps 2 and 3 for 2 times to replace culture medium completely.

4-4. (B) Live cell staining

Blocking

1) **Add 1 mL of blocking reagent** to the chamber.

2) **Discard 200 µL** from side ports.**(100 µL x 2 ports x 1 time)**

3) **Incubate** for 15 minutes at room temperature (for Protein block, incubation time will vary depending on the type of fixation reagent). Cover the chamber with the lid.

4) **Discard 800 µL** from side ports **(100 µL x 2 ports x 4 times)**.

Staining

5) **Add 1 mL of staining reagent** to the chamber.

6) **Incubate** by following time and temperature recommended for your staining solution.

Cover the chamber with the lid to avoid drying up the membrane.

For incubation over 1 h, recommend to incubate within humidified chamber to prevent drying up the membrane.

Washing

7) **Discard all liquid** from side ports. **(125 µL x 2 ports x 1 time)**

8) **Add 2 mL of 1 %BSA/PBS** to the chamber.

9) **Discard 2 mL** from side ports. **(200 µL x 2 ports x 5 times)**

10) **Repeat** steps 9 and 10 for 2 times.

11) **Add 1 mL of 1 %BSA/PBS** to the chamber.

Ready for imaging.

* If you need to stain with minimum amount of staining solution, please refer the later section : **4-4(C) Fixed Cell staining, Stainig, Option 2.**

4-4.(C) Fixed cell staining

Fixing

NOTE: Acetone cannot be used for fixation reagnet in SIEVEWELL.

1) **Add 500 µL of fixation reagent** to the chamber.

2) **Discard 100 µL** from side ports. Fixing reagent penetrate into nanowells by this operation.**(50 µL x 2 ports x 1 time)**

3) **Incubate** for 15 minutes at room temperature (for 4% PFA, incubation time will vary depending on type of fixation reagent). **Cover the chamber with the lid.**

Washing

4) **Discard 400 µL** from side ports. **(200 µL x 2 ports x 1 time)**

5) **Add 2 mL of PBS** to the chamber.

6) **Discard 2 mL** from side ports. **(200 µL x 2 ports x 5 times)**

7) **Repeat** steps 5 and 6 for 2 times.

Permeabilization

8) **Add 500 µL of permeabilization reagent** to the chamber.

9) **Discard 100 µL** from side ports. **(50 µL x 2 ports x 1 time)**

10) **Incubate** for 10 minutes at room temperature (for 0.2% TritonX-100/PBS, incubation time will vary depending on type of fixation reagent). **Cover the chamber with the lid.**

Washing

11) **Discard 400 µL** from side ports. **(200 µL x 2 ports x 1 time)**

12) **Add 2 mL of PBS** to the chamber.

13) **Discard 2 mL** from side ports. **(200 µL x 2 ports x 5 times)**

14) Repeat steps 12 and 13 for 2 times.

Blocking

15) **Add 1 mL of blocking reagent** to the chamber.

16) **Discard 200 µL** from side ports.**(100 µL x 2 ports x 1 time)**

17) **Incubate** for 15 minutes at room temperature (for Protein block, incubation time will vary depending on type of fixation reagent). Cover the chamber with the lid

18) **Discard 800 µL** from side ports.**(200 µL x 2 ports x 2 times)**

Staining

There is 2 options depends on the volume of staining solution.

Option1) Staining with a typical staining solution.

Staining

19) **Add 1 mL of staining reagent** to the chamber.

20) **Discard 200 µL** from side ports.**(100 µL x 2 ports x 1 time)**

21) **Incubate** by following time and temperature recommended for your staining solution. **Cover the chamber with the lid** to avoid drying up the membrane.

For incubation over 1 h, recommend to incubate within humidified chamber to prevent drying up the membrane.

Washing

22) **Discard 800 µL** from side ports.**(200 µL x 2 ports x 2 times)**

23) **Add 2 mL of PBS** to the chamber.

24) **Discard 2 mL** from side ports. **(200 µL x 2 ports x 5 times)**

25) **Repeat** steps 23 and 24 for 2 times.

26) **Add 1 mL of PBS** to the chamber.

Ready for imaging.

Option2) Staining with minimum amount of staining solution.

Staining

27) **Add 2 mL of 0.01% Tween20/PBS (=staining buffer)** to the chamber.

28) **Discard 2 mL** from side ports.**(200 µL x 2 ports x 1 time)**

29) **Carefully discard** all the residual liquid at the four corners by aspirating 20 µL from side ports (10 µL x 2 ports) for several times.

30) **Add 300 µL of staining reagent** to the chamber.

31) **Incubate** by following time and temperature recommended for your staining solution. **Cover the chamber with the lid** to avoid drying up the membrane.

For incubation over 1 h, recommend to incubate within humidified chamber to prevent drying up the membrane.

Washing

32) **Discard 220 µL** from side ports. **(110 µL x 2 ports x 2 times)** Not to aspirate too much liquid in chamber, aspiration volume is lower than added volume.

33) **Add 2 mL of PBS** to the chamber.

34) **Discard 2 mL** from side ports. **(200 µL x 2 ports x 5 times)**

35) **Repeat** steps 33 and 34 for 2 times.

36) **Add 1 mL of PBS** to the chamber.

Ready for imaging.