

WELLBAG

Operation Manual of Wellbag and the dedicated Holder

Preparation of materials

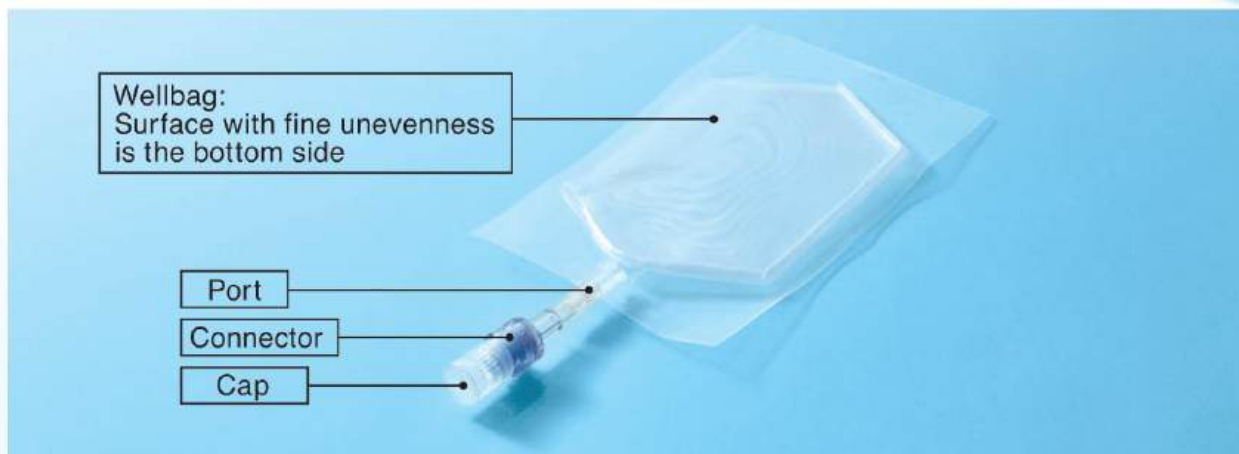


Fig.1 Overview of Wellbag and names of parts

- **Wellbag** (hereafter, “bag” is the same meaning) **Fig.1**
- **A dedicated holder of Wellbag** (hereafter “holder”)
- **A Syringe** : 20ml, screw lock type **Fig.2**
- **A pipettor** : recommend to use an electric pipettor and a 10mL tip
- **A centrifuge tube** : 15mL or 50mL
- **A suitable tube/container for collecting spheroids**
- **Cell suspension**
- **Culture medium** (hereafter “medium”)

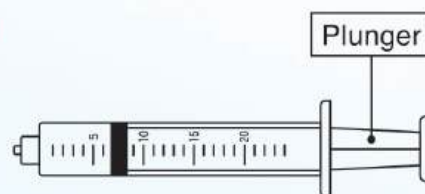


Fig.2

A Syringe :
20ml, screw lock type



Before start

Check the continuity of the connector

- 1 Remove the cap from the tip of the connector and connect the syringe with the plunger pulled about 5mL.
- 2 Push the plunger to inject a small amount air into the bag.
* If air does not enter, remove the syringe once and then screw it into the connector again.
- 3 Remove the syringe by pulling about 5mL of the plunger so as not to leave as much air in the bag as possible.

Procedures

01 Addition of medium into the Wellbag

- 1 Remove the plunger from the syringe and connect the syringe to the connector of the bag. **Fig.3**
- 2 Add 10ml of medium from the syringe to the bag using a pipettor. **Fig.4**
* At this time, pour the medium continuously so as not to involve air bubbles as much as possible.
- 3 While the syringe is connected to the connector, guide the air in the bag toward the port and push out it from the connector. **Fig.5**
- 4 After removing the large air bubbles, remove the syringe and attach the cap to the connector of the bag. **Fig.6**
* Insert the tip of the removed syringe into the centrifuge tube and attach the plunger to the syringe so that the medium does not scatter around.



Fig.3



Fig.4



Fig.5



Fig.6

02 Elimination of bubbles in microwells

Select one of the following two methods to expel the air bubbles remaining in microwells.

1 Manual method : air bubbles can be removed in a short time

- 1 With the cap attached to the connector, place the microwell side down and slam the bag horizontally onto workbench of the clean bench. **Fig.7, Fig.8**
- 2 Place the bag on the bench with the microwell side down, tap the top surface of the bag. **Fig.9**
- 3 Lift the bag and visually check if there are any air bubbles left. If bubbles left, tap the area with a finger. **Fig.10**



Fig.7



Fig.8



Fig.9



Fig.10

- ④ Remove the cap from the connector and connect the syringe with the plunger pushed in.
- ⑤ Move small air bubbles floating on the upper surface of the bag to the connector side.
- ⑥ While holding the rear to the center of the bag by hand, make the port stand upright and guide the air bubbles to the port side. **Fig.11, Fig.12**
- ⑦ After the air bubbles have sufficiently sent to the port side, pull the plunger of the syringe gradually to expel them.
- ⑧ Remove the syringe and attach the cap to the connector.



↓ **Fig.11**



Fig.12

② Incubation method : it takes about half day to expel the air bubbles

Set the bag in the holder by the method described in P.3, **04 “Setting a Wellbag on the dedicated holder”**, and by the overnight incubation at 37°C in an incubator, air bubbles in the bag are expelled outside of the bag naturally.

03 Addition of cell suspension into the bag

- ① Remove the connector cap and connect the syringe with the plunger removed to the connector.
- ② By the same procedure as P.1, **01 “Addition of medium into the Wellbag”**, the cell suspension is injected into the bag with a pipetter.
 - *Inject the cell suspension just below the port.
 - *The total amount of medium and cell suspension in the bag shall be within 20ml.
- ③ Expel air bubbles in the bag by the method described in P.1, **01, ③ or 02, 1**.
- ④ Attach the cap to the connector and place the bag flat with the microwell side facing up.
- ⑤ Stir the cell suspension in the bag while taking care not to foam the medium.

〈Example of stirring method〉

- ① Push the bag left and right alternately from above by using both hands. **Fig.13**
- ② Lift the bag and shake it left and right on the palm with the microwell side facing up. **Fig.14**

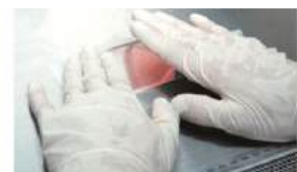


Fig.13



Fig.14

***After stirring the cell suspension, proceed to the next section, “Setting a Wellbag on the dedicated holder” immediately.**

04 Setting a Wellbag on the dedicated holder

- 1 Open the upper lid(movable part)of the dedicated holder upwards.
- 2 Put the bag in the center of the holder plate aligning it with the edge of the bottom glass, then close the upper lid slowly.

Fig.15 - Fig.17

* Confirm there are no wrinkles on the top surface of the bag. If the surface is wrinkled, smooth the surface by the hand and close the lid again.

* Confirm that the lock of the upper lid of the holder is working properly.

- 3 Keep it gently at room temperature for 5 to 10 minutes. Cells will fall into the microwells.

Notes for carrying a holder with a bag set:

- Confirm that the lock of the upper lid of the holder is working properly.
- Handle the holder slowly so that the spheroids do not move between microwells.
- Keep the holder horizontal as much as possible and don't make a sudden turn during transportation.



Fig.15



Fig.16



Fig.17

05 Culture of cells

A Wellbag containing cell suspension attached to the holder is placed in an incubator to culture cells for spheroids formation.

* Please set the incubation conditions such as temperature and time according to the own purpose.

06 Recovery of spheroids

- 1 Take out the holder from the incubator.
- 2 Remove the bag from the holder and lay it with the microwell surface above on the workbench in the clean bench.
- 3 Tap the bag lightly from above so that spheroids leave from microwells.
- 4 Remove the cap from the connector of the bag.
- 5 Attach the syringe to the connector while pulling the plunger about 15mL.
- 6 Push the plunger completely to inject about 15mL of air into the bag. **Fig.18**

* Operate slowly so that the injected air makes one big bubble.

- 7 Hold the bag with the port-connector side down, and aspirate spheroids with a syringe. **Fig.19**

* Aspirate the contents carefully so that the top and the bottom films of the bag do not stick together.

* Collect the spheroids by shaking the cell suspension to wash away the microwell surface of the bag.

- 8 Transfer the recovered spheroids to a collection tube/container.

Fig.20



Fig.18



Fig.19



Fig.20

Note: This manual shows an example of the procedures for using a Wellbag and a dedicated holder recommended by our company and does not prohibit original methods and procedures performed under the responsibility of the users. Also, using Wellbags according to this manual does not guarantee the effectiveness of this product.