GenoStaff

Reagents Enabling Preparation Of Gelled Cell Suspension

iPGell®

(20-Test Kit)

<< Product Manual>>

Product Number: PG20-1

Lot Number: See labels of outer case and each reagent tube

Product: Kit for solidifying various types of cell suspensions including cultured cells, blood cells, 3D culture cells and extremely small samples.

Shipment: Shipped frozen in dry ice (-20ºC)

Storage: Store reagents (A-solution and B-solution) at -20ºC immediately upon arrival.

Do not store in frost-free freezer with automatic thaw-freeze.

Components of Kit:

Reagents: A-solution ・・・ 4 tubes (One tube contains 60 µL of solution; 50 µL for 5 tests and extra 10 µL for practice)

B-solution ・・・ 4 tubes (One tube contains 300 µL of solution; 250 µL for 5 tests and extra 50 µL for practice)

Accessories: 24 Sample Tubes (Four of them are for practice)

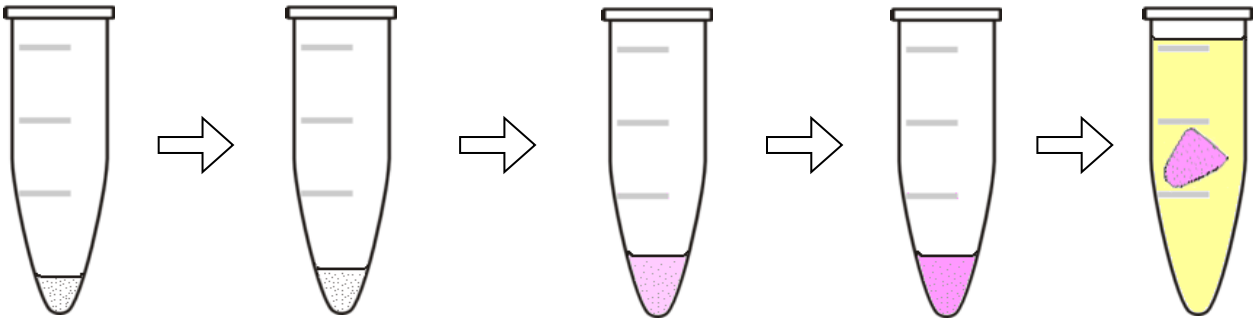
Product Manual

Warranty: One year after receiving the product.

The warranty period expires on the expiry date of the product.
Warranty could be invalidated if the instructions in this manual are not followed.

*** Preparation ***

< Preparation Flow >



50 µL of Cell Suspension

Add 10µL of A-Solution and mix well by pipetting up and down

Add 50µL of B-Solution and pipette up and down immediately

Leave at room temperature for 1 minute to allow the mixture to solidify

Add 1.3 mL of fixative solution

Try how fast the solution solidifies using medium or buffer prior to the experiment.

[Dispersed Cells such as Cultured Cells and Blood Cells]

Steps:

1. Place A-solution on ice and B-solution at room temperature after thawed. *1
2. Centrifuge and resuspend cells in a small amount of medium or buffer, and then place the cell suspension on ice. *2, 3
3. Mix 10 µL of A-solution and 50 µL of cell suspension in a provided sample tube and pipette the mixture up and down.
4. Add 50 µL of B-solution (in red) into the sample tube and pipette the mixture up and down 3 times immediately.

- Pipette the mixture up and down immediately since the mixture solidifies within seconds.
- Do not pipette the mixture 4 or more times.
  Over-pipetting causes the mixture to solidify at the tip of pipette.
- Do not tap, vortex or shake the sample tube after the B-solution is added.
- Do not break bubbles if bubbles are formed during pipetting. Leave the bubbles as they are.

5. Leave the sample tube at room temperature for one minute and make sure that the mixture has been solidified into a gel by turning the sample tube upside down. (See Photo 1)
6. Add a fixative solution into the sample tube and gently pipette for the gel to come off the sample tube. (See Photo 2) *4
7. Fix the gel with appropriate fixative solution for the desired staining method and prepare a tissue block.

*1 Thaw the reagents promptly at 37ºC and mix well after thawed.
The B-solution is colored red with Phenol red for visibility of the gel in the fixative solution.
The color of B-solution may turn to yellow due to pH of cell suspension or storage conditions. There is no problem with the quality.
When a precipitate is seen in the B-solution, lightly centrifuge the B-solution and use its supernatant.

*2 See Image 1 for an indication of cell concentration for resuspension.
Medium or neutral pH buffer is suitable as a solution to resuspend cells. There is no problem if the medium contains serum.

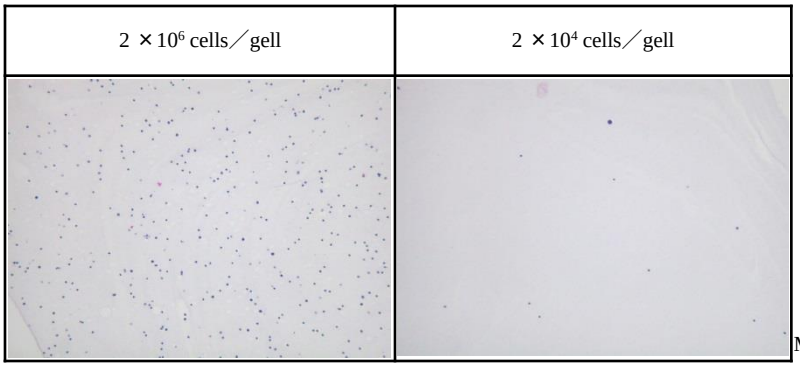
*3 When using fixed cells, the cell suspension may not solidify completely if the fixative solution remains in the sample tube. In this case, remove the fixative solution by light centrifugation, wash the cells with PBS, and then resuspend the cells in medium or PBS.
After the cell suspension solidify into a gel, immerse the gel into the fixative solution again.

*4 Use the appropriate fixative solution according to the desired staining method, such as ISH or immunohistochemical staining. We recommend a kind of fixative solution of formalin series for ISH.
The color of the gel turns to transparent from red during fixation.
This product is not suitable for acetone fixation.

[Cell Clusters such as Extremely Small Tissue and 3D Culture Cells (e.g., Embryoid Bodies)]

Use a pipette with cut-off tip when pipetting the sample solution to avoid damaging tissue structures and cell clusters. (Steps 3 and 4) The tip of pipette can be cut off with scissors or the like. (See Photo 3)

< Image 1: Indication of Cell Concentration >



Magnification 100×

< Photos >

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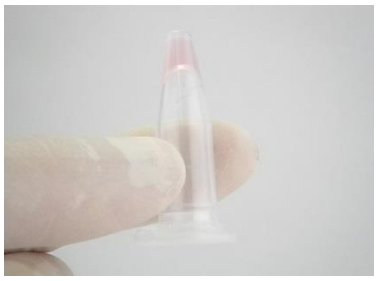

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Caution:

- The reagents (A-solution and B-solution) cannot be frozen again once the reagents are thawed.
- Always use gloves when handling the reagents.
- This product is for research purpose only. This product is NOT for medical use, food, cosmetics or household appliances.

[Using Samples Prepared with iPGell as Paraffin Blocks]

<Paraffin Block Preparation Steps>

1. Prepare a gel of cell suspension using iPGell and immerse the gel into a fixative solution.
   (See the previous page for instruction)
2. Shake the sample tube gently and leave the sample tube overnight to all day for fixation.
3. Rinse the gel with PBS at once, and then wash with fresh PBS for 30 minutes
4. Replace the PBS with 70% ethanol – 30 minutes × 2
5. Proceed to paraffin-embedding using a standard method (70% ethanol -)

*The gel can be stored about 3 days at 4ºC for after the PBS is replaced with 70% ethanol.
After the paraffin-embedding process, the size of the gel shrinks to 70 – 80% of its original size.

- Examples of Paraffin-Embedding using Automatic Rotary Tissue Processor -

1. 80% ethanol Room Temp. 1 hr.
2. 90% ethanol Room Temp. 1 hr.
3. 100% ethanol 1 Room Temp. 1 hr.
4. 100% ethanol 2 Room Temp. 1 hr.
5. 100% ethanol 3 Room Temp. 1 hr.
6. 100% ethanol/xylene 1:1 Room Temp. 1 hr.
7. Xylene 1 Room Temp. 1 hr.
8. Xylene 2 Room Temp. 1 hr.
9. Xylene 3 Room Temp. 1 hr.
10. Paraffin 1 65ºC 1 hr.
11. Paraffin 2 65ºC 1 hr.
12. Paraffin 3 65ºC 1 hr.

Caution:

The gel prepared using iPGell may be deformed if a tissue processor with pressurization/depressurization system is used during paraffin-embedding process. Accordingly, rotary tissue processor is recommended.

[Using Samples Prepared with iPGell as Fixed-Frozen Block

<Fixed-Frozen Block Preparation Steps>

1. Prepare a gel of cell suspension using iPGell. (See the previous page for instruction)
2. Add 4%PFA/PBS and leave the gel for one hour to overnight for fixation.**[*1]**
3. Rinse the gel with PBS at once, and then wash with fresh PBS for 30 minutes
4. Transfer the gel to a cassette or the like for sucrose replacement (4ºC)**[*2]**
   4-1. 10% sucrose/PBS 2-4 hrs.
   4-2. 20% sucrose/PBS 2 hrs. to overnight
   4-3. 30% sucrose/PBS 2 hrs. to overnight
5. Roll the gel on a paper towel after the sucrose replacement is completed to remove the moisture on the gel.**[*3]**
6. Lightly soak the gel in a small volume of compound first, for 15 minutes.
7. Fill a cryomold with fresh compound, and embed the gel mentioned in step 6 above.
8. Quick-freeze the compound containing the gel with dry ice/hexane.**[*4]**

*1 For immunohistochemical staining, the optimum time for fixation depends on the antibody that is used.
- During fixation, gently shake the sample tube using a shaker.
- When using granule cells, leave the gel overnight for fixation because the granule cells are easily damaged.

*2 Transfer the gel into a larger container from the sample tube before sucrose replacement.
- Since the gel is hard to sink into the sucrose solution, the gel must be placed in a cassette or pressed down using paper towel to sink into the sucrose solution, during sucrose replacement. (See Photos 4 and 5)
- Take sufficient time for sucrose replacement to remove the fixative solution completely. The stainability may be affected by the remaining of fixative solution.

*3 Do not allow the gel to dry.

*4 Caution must be taken when using liquid nitrogen; liquid nitrogen may cause the gel to crack.

Caution: Preparation for Fixed-Frozen Section

- The gel in a block is hard to see in the fixed-frozen block.
- The temperature must be set to -25ºC when the block is sectioned. When the temperature is high, the gel portion in the block adheres to the knife, and sections may not be made properly.
- Caution must be taken when handling the section since the gel portion is easily torn.
- Use adhesive slides for preparing sections.

  Following slides are recommended:

  Poly-l-lysine (PLL)-coated slides (Muto Pure Chemicals Co., Ltd.)

  APS coat slides (Matsunami Glass Ind., Ltd.)
- Use of chilled slide may cause the gel portion to shrink when attaching sections to the slide. 3D culture cells and extremely small tissues may be deformed as the gel shrinks.

  So, use room temperature slide when attaching sections.
- Some slides, depending on the type and manufacturing lot of slides, may cause the gel portion to greatly shrink.
- Store the frozen block at -80ºC.

  Frequent temperature changes between -80ºC to -20ºC may cause the block to deteriorate.

This product is not for fresh frozen block and section.

< Photos >

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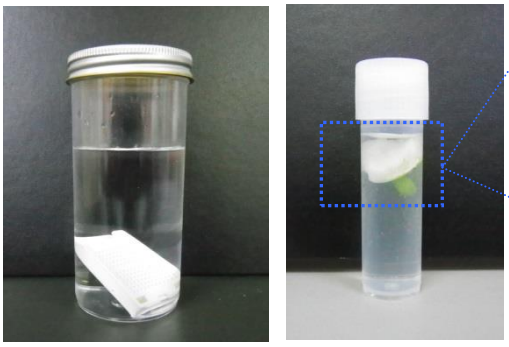

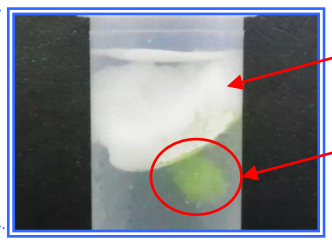

*The gel is colored yellow for demonstration purposes.
The actual color of the gel is transparent.

≪Inquiry about this product, please contact us.≫

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