GenoStaff

～The Kit for a new type smear preparation～

# *Smear Gell* ™

《 Manual ～brief version～》

～ Please read this manual before use ～

≪Any inquiries about this product≫

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## § 1. Preparation

### 1. Sample

1) To prepare cell suspension, re-suspend cells in small amount of cell culture medium or neutral buffer after centrifugation.

＊ Fig. 1 is rough standard for concentrations of cell suspension. If the suspension looks to have enough conc. (approx. 4 x $10^3$ cells/μL), it is unnecessary to carry out centrifugation and re-suspension.

＊ When the cell sample is fixated, centrifuge it and discard the fixative. After washing the cells with PBS, re-suspend with mediums or buffers.

Re-fix the smear preparation with an appropriate fixative, after solidifying the sample on slideglass.

＊ When the cell suspension contains cryoprotectant like ñCell Bankerò or Glycerol, wash the cells and re-suspend with mediums or buffers.

2) Prepare 3μL of cell suspension per one slideglass.

＜Table-1 Volume of mixture (cell suspension and solution Ⅰ) required for each number of slide ＞

| | | Number of slideglass | | | | |
|---|---|---|---|---|---|---|
| | | one | two | three | five | ten |
| Use as mixture | Cell suspension | 3 μL | 6 μL | 9 μL | 15 μL | 30 μL |
| | Solution Ⅰ | 2 μL | 4 μL | 6 μL | 10 μL | 20 μL |

*Use 5μL of solution Ⅱ per one slideglass

### 2. Reagents and Others

1) Thaw reagents (solution Ⅰ, Ⅱ) of the Smear Gell kit in hands, just before to use and keep them at room temperature.

＊ Solution Ⅰ and Ⅱ are able to repeat only 2 ~ 3 times FREEZE-THAW cycles.

2) Prepare the APS coated slideglasses (MATSUNAMI).

＊ If you use slideglasses of kit attachment, return them to room temperature before use.

3) Prepare an appropriate fixative.

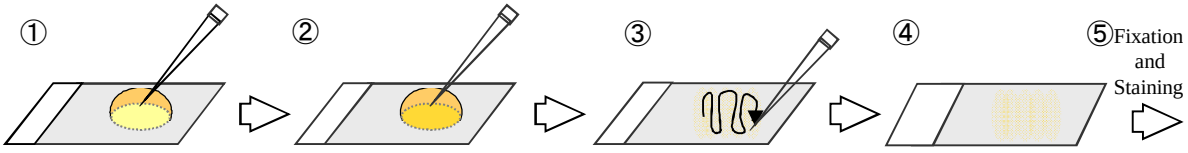
＊ When you fix smear preparations using this kit on slideglasses, soak them whole in the fixative.

＊ Choose appropriate fixative suitable for your staining method. DO NOT USE ACETONE for this kit.

## § 2. Method of preparing smear preparation using Smear Gell

【Preparing only one slideglass at once】

＜Schematic＞




① Put 3 µL of cell suspension on a slideglass.

② Add 2 µL of solution Ⅰ to the cell suspension and mix by pipetting on the slideglass.

③ Add 5 µL of solution Ⅱ to the mixture on the slideglass, and **pipetting 5 times immediately after.** Then smear the total mixture thinly on the slideglass **using same tip and in a series of actions.**

④ Leave the slideglass at room temperature for 2 min. DO NOT DRY UP.

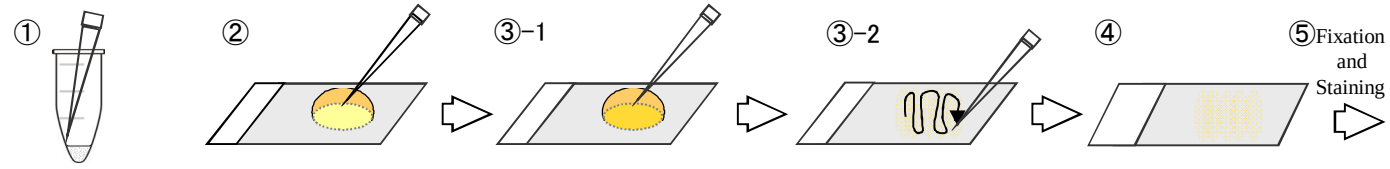
⑤ Fixation and Staining

【Protocol】

1. Put 3 µL of cell suspension on a slideglass.
2. Add 2 µL of solution Ⅰ to the cell suspension and mix by pipetting on same slideglass.
3. Add 5 µL of solution Ⅱ to the mixture on same slideglass, and pipetting 5 times immediately after, and furthermore smear the total mixture thinly with same tip, in a series of actions. It is easier to smear that the tip is slanted like Fig. 3.
   * Do not pipetting more than 5 times when you mix ñmixture Aò and solution Ⅱ, and start smearing no sooner after finish 5 times pipetting.
   * If you feel difficulty in smearing with normal tips, cut the head of tips like Fig. 2 and try to use it in Step 3.
4. Leave the slideglass at room temperature for 2 minutes to solidify the gel. AVOID DRYING.
   * Do not leave more than 2 minutes.
5. Soak the slideglass in an appropriate fixative which suitable for your objective staining method.
   * About 15 ~ 30 minutes fixation is enough.

【Preparing some slideglasses at once】

＜Schematic＞




Add solution Ⅰ to the cell suspension and pipetting.

(ex) for 5 slides
cell suspension : 15 µL
solution Ⅰ : 10 µL

Put 5 µL of solution Ⅱ on a slideglass.

Add 5 µL of mixture (①) to the solution Ⅱ on the slideglass, and **pipetting 5 times immediately after.**

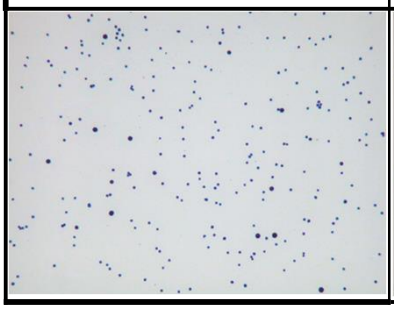
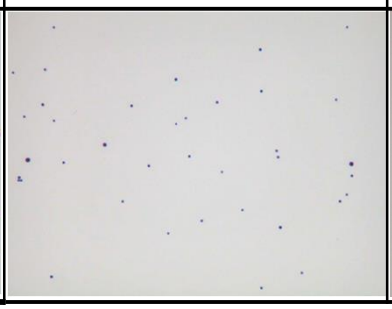
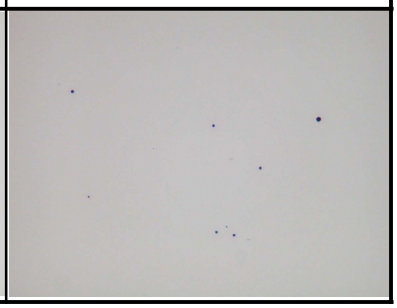
Smear the total mixture thinly on the slideglass **using same tip and in a series of actions.**

Leave the slideglass at room temperature for 2 min. DO NOT DRY UP.

【Protocol】

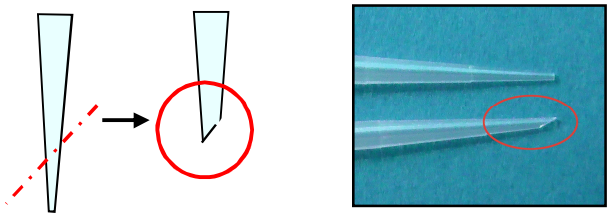
1. Add solution Ⅰ to the cell suspension and pipetting well. (Ÿ mixture A)
   ＊ See Table-1
2. Put 5 µL of solution Ⅱ on a slideglass.
3. Add 5 µL of mixture A to solution Ⅱ on the slideglass, and pipetting 5 times immediately after, and furthermore smear the total mixture thinly with same tip, in a series of actions. It is easier to smear that the tip is slanted like Fig. 3.
   ＊ Do not pipetting more than 5 times when you mix ñmixture Aò and solution Ⅱ, and start smearing no sooner after finish 5 times pipetting.
   ＊ If you feel difficulty in smearing with normal tips, cut the head of tips like Fig. 2 and try to use it in Step 3.
4. Leave the slideglass at room temperature for 2 minutes to solidify the gel. AVOID DRYING.
   ＊ Do not leave more than 2 minutes.
5. Soak the slideglass in an appropriate fixative which suitable for your objective staining method.
   ＊ About 15 ~ 30 minutes fixation is enough.

＜Fig. 1 Rough standard for concentration of cell suspension ＞

| 7.5 x $10^4$ cells / slide<br>(= 2.5 x $10^4$ cells /μL) | 1x$10^4$ cells / slide<br>(= 3.3 x $10^3$ cells /μL) | 1x$10^3$ cells /slide<br>(= 3.3 x $10^2$ cells /μL) |
|---|---|---|
|  |  |  |

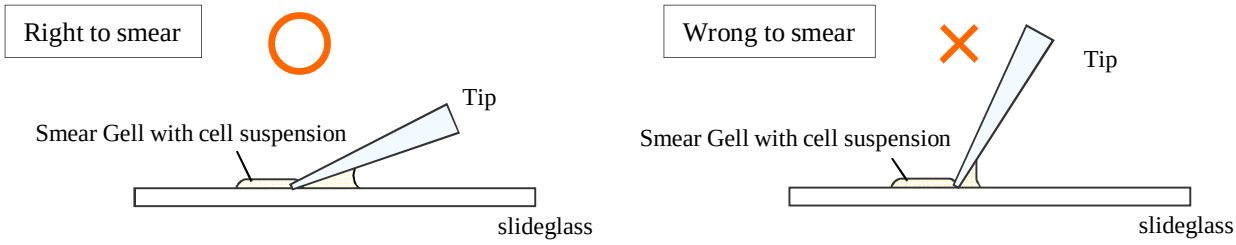
Hematopoietic cells (almost leukocyte) mag. x 100

＜Fig. 2 Tip＞

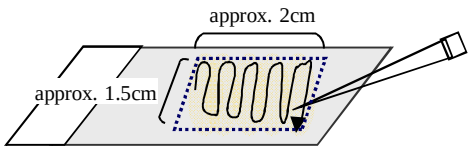


＜Fig. 3 Point of smearing ＞

《SIDE VIEW》




《OVERHEAD VIEW》

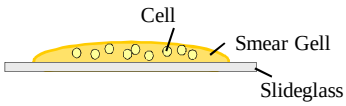


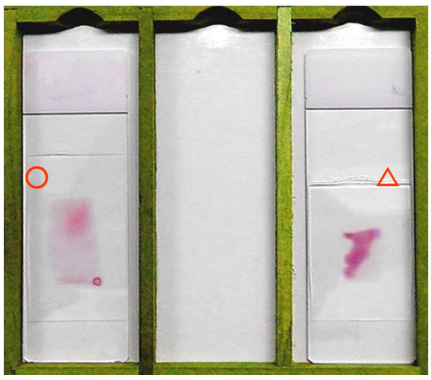

It is suitable for smearing at appropriate thickness to make the tip shuttle about 5 times in approx. 2cm×1.5cm area.
DO NOT CARRY OUT RE-SMEARING.
Finish smearing in one action.

＜Fig. 4 Example of smear preparation using Smear Gell＞

○

＊ Uniformly spreaded and appropriate thick Smear Gell containing cells.
＊ Cells were almost monolayer.
＊ It is easy to observe by a microscope.

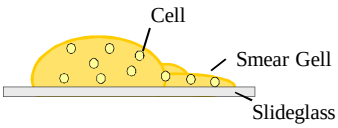


HE stain

△

＊ Smear Gell is likely to unstick due to its inequality.
＊ Cells were multi-layer.
＊ It seems difficult to observe by a micro-scope.



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