GenoStaff

～The Kit for a new type smear preparation～

Smear Gell®

～ Please read this manual before use ～

Product Number: SG-01

Lot Number: See labels of 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 (I-solution and II-solution) at -20ºC immediately upon arrival.
Do not store in frost-free freezer with automatic thaw-freeze.

Components of Kit:

Reagents: I-solution (One tube - 25 µ×4 for 40 tests including extra 4 µL for trial)
II-solution (One tube – 60 µL×4 for 40 tests including extra 10 µL for trial)

Accessories: 40 Slides
Product Manual

Warranty: One year after receiving the product.

≪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 × 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 “Cell Banker” 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 solutionI) 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 solutionIIper one slideglass

2. Reagents and Others

1) Thaw reagents (solutionI, 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).

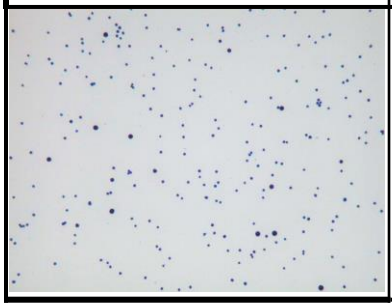
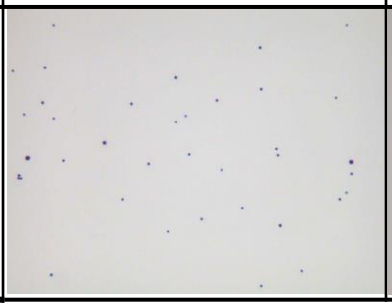
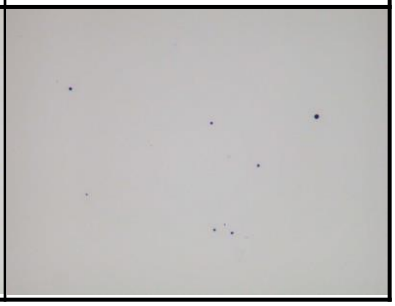
＊ If you use slideglasses of kit attachment, return them to room temperature before use.

3) Prepare an appropriate fixative.

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

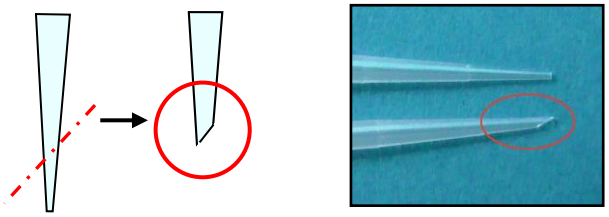
＊ Choose appropriate fixative suitable for your staining method. DO NOT USE ACETONE for this kit.

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

7.5×10^4 cells / slide (= 2.5×10^4 cells /μL)	1×10^4 cells / slide (= 3.3×10^3 cells /μL)	1×10^3 cells /slide (= 3.3×10^2 cells /μL)
		

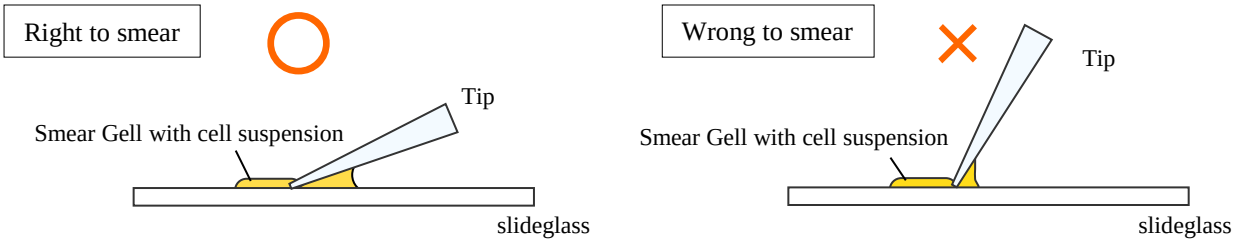
Hematopoietic cells (almost leukocyte) mag. × 100

＜Fig. 2 Tip＞

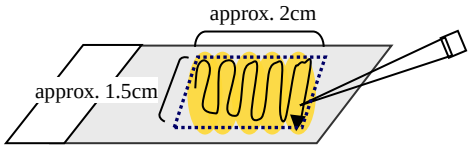


＜Fig. 3 Point of smearing ＞

《SIDE VIEW》



《OVERHEAD VIEW》

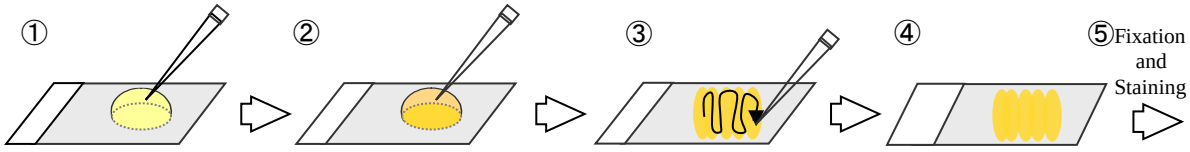


It is suitable for smearing at appropriate thickness to make the tip shuttle about 5 times in approx. 2cm×1.5cm area.
DO NOT CARRY OUT RE-SMEARING.
Finish smearing in one action.

§ 2. Method of preparing smear preparation using Smear Gell

【Preparing only one slideglass at once】

＜Schematic＞



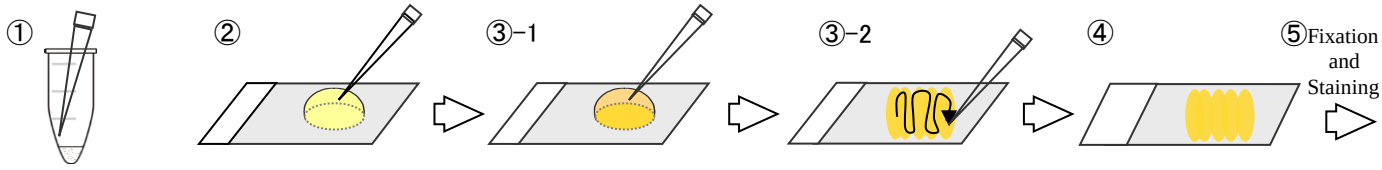
①	②	③	④
Put 3 µL of cell suspension on a slideglass.	Add 2 µL of solutionI to the cell suspension and mix by pipetting on the slideglass.	Add 5 µL of solutionII to the mixture on the slideglass, and **pipetting 5 times immediately after.** Then smear the total mixture thinly on the slideglass **using same tip and in a series of actions.**	Leave the slideglass at room temperature for 2 min. DO NOT DRY UP.

【Protocol】

1. Put 3 µL of cell suspension on a slideglass.
2. Add 2 µL of solutionI to the cell suspension and mix by pipetting on same slideglass.
3. Add 5 µL of solutionII 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 “mixture A” and solutionII, 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 solutionI to the cell suspension and pipetting.

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

Put 5 µL of solutionII on a slideglass.

Add 5 µL of mixture (①) to the solutionII 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 solutionI to the cell suspension and pipetting well. (→ mixture A)
   ＊ See Table-1
2. Put 5 µL of solutionII on a slideglass.
3. Add 5 µL of mixture A to solutionII 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 “mixture A” and solutionII, 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. 4 Example of smear preparation using Smear Gell＞

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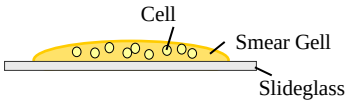
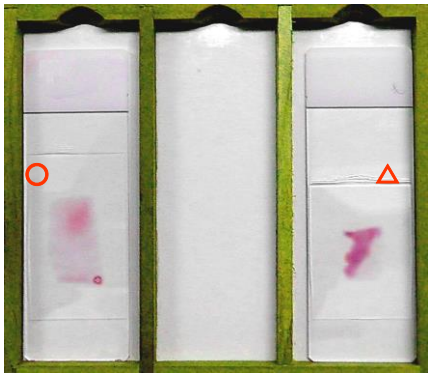
＊ Uniformly spreaded and appropriate thick Smear Gell containing cells.
＊ Cells were almost monolayer.
＊ It is easy to observe by a microscope.





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.