Giemsa Staining Solution(10X)

Catalogue No.: K088

Size: 100mL

Kit component:

Item	100mL
Reagent A: Giemsa Staining Solution(10X)	10 mL
Reagent B: Phosphate buffer	100 mL

Required Instruments and Reagents：

1. Slide, Microscope
2. Distilled water
3. Methyl alcohol
4. 0. 1～0.5% acetic acid

Storage:

The kit should be stored at 25° C for one year.

Principle of the Assay:

Giemsa Staining Solution is a mixture of azurin II and eosin. The principle and results of Giemsa staining are basically the same as Wright's staining. The Giemsa staining solution had a strong staining effect on the cytoplasm, which could better show the basophilic degree of the cytoplasm. Especially for the azurophilic, eosinophilic and basophilic granules in blood and bone marrow cells, the staining was clear, but the staining was darker for the nuclei, and the staining of the nuclear structures was not good. Therefore, Giemsa staining solution is often used in combination with Wright's staining solution.

Giemsa Stain was prepared by grinding with imported Giemsa pigment, methanol, and special contrast dye, which could show clear cell staining effect. It is often used for staining of tissue cuts, blood and cell smears, bacteria, chromosome banding, protozoan parasites, etc.

The eosinophilic granules were basic proteins. Eosinophilic particles combine with the acid dye eosin and are stained pink and are referred to as eosinophils. Nuclear proteins and lymphocyte cytoplasm were acidic. Nuclear proteins and lymphocyte cytoplasm are stained violet blue in combination with the basic dyes methylene blue or azulean and are called basophilic substances. The neutral particles are in an isoelectric state. Neutral particles can be combined with eosin and methylene blue, and are stained with light purple, which is called neutral material.

This staining solution consisted of 10× storage solution and phosphate buffer. 1:9 mixed into a working solution after use. It can also be used separately, that is, first stained with Giemsa Stain, and then treated with phosphate buffer solution, also can obtain satisfactory staining results.

Assay Procedure (For reference):

一. Smear staining was performed in one step

1. Preparation of Giemsa working solution：

The reagent was mixed as reagent A: reagent B=1:9. The resulting reagents can be used directly. Cannot be stored for a long time.

2. Blood smears or bone marrow smears were prepared using conventional methods. After the smear was allowed to dry naturally, it was fixed with methanol for 1 to 3min.

2. A blood smear or bone marrow smear was placed on a staining rack, and Giemsa working solution was dropped to cover the smear. The stain was allowed to drip over the sample for 15 to 30min at room temperature.

3. Rinse slowly from one end of the slide with tap or distilled water.

4. The samples were dried and examined by microscopy.

二. Smear staining was performed in two step

1. Preparation of Giemsa working solution：

The reagent was mixed as reagent A: distilled water=1:4， The resulting reagents can be used directly. Cannot be stored for a long time.

2.Blood smears or bone marrow smears were prepared using conventional methods. After the smear was allowed to dry naturally, it was fixed with methanol for 1 to 3min.

3. A blood smear or bone marrow smear was placed on a staining rack, and Giemsa working solution was dropped to cover the smear. The stain was allowed to drip over the sample for 15 to 30min at room temperature.

4. An equal volume of phosphate buffer was added, and the slides were gently shaken and allowed to stand at room temperature for 5 to 10min.

5. Rinse slowly from one end of the slide with tap or distilled water.

6. The samples were dried and examined by microscopy.

三. Staining of tissue sections

1. Preparation of Giemsa working solution：

The reagent was mixed as reagent A: reagent B=1:9. The resulting reagents can be used directly. Cannot be stored for a long time.

2. Fresh tissues were immediately fixed in Regaud's fixative for 2 days, during which time the fixative should be changed once。

3. The plates were fixed with 3% potassium dichromate for 1 day.

4. Rinse with running water for 16 hours or overnight.

5. Samples were routinely dehydrated and embedded。

6. Sections were approximately 5 μ m thick and were routinely deparaffinized to water。

7. They were then washed twice with distilled water for 1min each time.

8. The samples were placed into a dye tank containing Giemsa working solution for 18 to 24h and then washed slightly with distilled water.

9. Samples were washed with 0. 1 to 0.5% acetic acid for 1 to 2min and then slightly rinsed with tap water.

10. Samples were rapidly dehydrated with absolute ethanol three times for 5 to 10s each time.

11. Clear in xylene or deparaffinized clear solution and sealed in neutral resin.

Results:

Eosinophilic granules	Pink
Basophilic granules	Violet blue
Neutral granules	Light purple

Notes:

1. The blood smear or bone marrow smear should be uniform in thickness so as not to affect the staining effect.

2. Smear staining after Giemsa staining, do not remove the staining solution first or wash the smear vigorously directly.

3. If the staining is too deep or too light, the staining time or concentration of the working solution should be adjusted.

4. In smear staining and tissue section staining, the pH value has a certain effect on staining, and the slides should be clean and free of acid and base contamination to avoid affecting the staining effect.

5. If the liquid level of the staining solution should be metallic luster after dilution, it indicates that the staining solution has staining effect, otherwise the staining solution may be ineffective。

6. In the staining of tissue sections, a large amount of 0. 1～0.5% acetic acid should be used to wash the sections quickly after staining to avoid the contamination of floating sediment.

7. 0.5% acetic acid differentiation is often used for Giemsa staining of tissue sections, and can also be used for cell smears if necessary, but its concentration should be reduced. When sections were differentiated with 0.5% acetic acid, they were terminated when they were pink.

8. In Giemsa tissue section staining, dehydration with absolute ethanol should be rapid, otherwise the section is easy to fade.

9. In smear staining and tissue section staining, if the results need to be obtained quickly, the Giemsa working solution can be prepared according to the storage solution (10×): phosphate buffer solution =1:1, thoroughly mixed, that is, the rapid Giemsa staining working solution, the staining solution is added to the cell smear or tissue section, heated staining, and the staining solution is added again after 20 ~ 30s. The procedure was repeated 5 to 10 times, and the rest of the procedure was the same as above。

10. The staining solution can be reused, but it should not be repeated many times. If there is sediment, it should be filtered and used.

11. Regaud fixative: prepared as 3% potassium dichromate: formaldehyde =4:1, mixed before use, expired after 1 to 2 days.

12. The reagent is only suitable for scientific research, not for clinical diagnosis or other purposes.