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Giemsa staining

Product Number: BCGM377-100ml

Component

Component	BCGM377-100mL
Giemsa staining A: 10×Giemsa stain Buffer	10mL
Giemsa staining B: Phosphate buffer	100mL

Description

Jimsa pigment (also known as Jimsa pigment) is a mixture of azurol II and eosin. The principle and results of Giemsa staining are basically the same as those of Wright staining. Giemsa staining solution has strong staining power on the cytoplasm and can better display the alkaline degree of the cytoplasm, especially for azurophilic, eosinophilic, and basophilic particles in blood and bone marrow cells. The staining is clear, but the staining of the nucleus is darker and the nuclear structure is poorly colored. Therefore, Giemsa staining solution is often used in combination with Wright staining solution.

Giemsa staining is mainly made from imported Giemsa pigments and methanol, containing Bomei's unique staining agent. It is prepared by grinding and can present clear cell staining effects. It is often used for staining tissue sections, blood and cell smears, bacteria, chromosome banding, protozoan parasites, etc. Eosinophilic particles are alkaline proteins that bind to the acidic dye eosin and turn pink, known as acidophilic substances; Nuclear proteins and lymphocyte cytoplasm are acidic and bind to alkaline dyes such as methylene blue or azurol, staining purple blue and called alkaline substances; Neutral particles are in an isoelectric state and can bind to both eosin and methylene blue, dyeing a light purple color, and are called neutral substances.

Giemsa staining (1:9) is composed of 10 x storage solution and phosphate buffer solution, which are mixed into a working solution at a ratio of 1:9 before use; It can also be used separately, by first staining with Giemsa Stain and then treating with phosphate buffer solution, satisfactory staining results can be obtained. This reagent is only used in the field of scientific research and is not suitable for clinical diagnosis or other purposes.

Required materials

1. Glass slides, microscopes;
2. Distilled water, methanol, 0.1-0.5% acetic acid.

Protocol

1. One step smear staining

1.1. Configuration of Giemsa working fluid:

Mix reagent (A): reagent (B)=1:9, that is, take 1 part of Giemsa Stain storage solution (10 ×) and add it to 9 parts of phosphate buffer solution, mix well, which is Giemsa working solution. It is a ready to use reagent that is not easy to store and can be prepared immediately.

1.2. The commonly used method is to prepare blood or bone marrow smears. After the smear is naturally dried, fix it with methanol for 1-3 minutes.

1.3. Place the blood or bone marrow smear on the staining rack, cover the smear with Giemsa working solution, and stain at room temperature for 15-30 minutes.

1.4. Slowly rinse one end of the glass slide with tap water or distilled water. Dry and undergo microscopic examination.

Staining results:

Eosinophilic granules	Pink
Alkaline granules	Purplish blue
Neutral particles	Lavender

2. Two-step smear staining

2.1. Configuration of Giemsa working fluid:

Mix reagent (A): distilled water=1:4, that is, take 1 part of Giemsa Siain storage solution (10 ×) and add it to 4 parts of distilled water to mix well, which is the Giemsa working solution. Giemsa working solution is a ready to use reagent that is not easy to store and can be prepared immediately.

2.2. The commonly used method is to prepare blood or bone marrow smears. After the smear is naturally dried, fix it with methanol for 1-3 minutes.

2.3. Place the blood or bone marrow smear on the staining rack, cover the smear with Giemsa working solution, and stain at room temperature for 15-30 minutes.

2.4. Add an equal amount of phosphate buffer solution, gently shake the glass slide, and let it stand at room temperature for 5-10 minutes.

2.5. Slowly rinse one end of the glass slide with tap water or distilled water. Dry and undergo microscopic examination.

Staining results:

Eosinophilic granules	Pink
Alkaline granules	Purplish blue
Neutral particles	Lavender

3. Tissue section staining

3.1. Preparation of Giemsa working solution: Mix reagent (A): reagent (B)=1:9, that is, take 1 part of Giemsa Stain storage solution (10 ×) and add it to 9 parts of phosphate buffer solution, mix well, and it is Giemsa working solution; Giemsa working solution is a ready to use reagent that is not easy to store and can be prepared immediately.

3.2. Fresh tissue should be immediately placed in Regaud fixative for 2 days, during which the fixative should be replaced once.

3.3. Fixed with 3% potassium dichromate for 1 day.

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

3.5. Conventional dehydration and embedding.

3.6. The slice thickness is about 5 μ m, and it is commonly dewaxed to water.

3.7. Wash twice with distilled water, each time for 1 minute.

3.8. Immerse in Giemsa working solution for 18-24 hours in a dyeing tank, and clean slightly with distilled water.

3.9. Wash with 0.1-0.5% acetic acid for 1-2 minutes, then rinse slightly with tap water.

3.10. Dehydrate rapidly with anhydrous ethanol three times, each time for 5-10 seconds.

3.11. Xylene or Bomei dewaxing transparent liquid is transparent and sealed with neutral resin.

Staining results:

Nucleus	Blue to purple
Cytoplasm	Light blue
Chromaffin cell satiety	Yellow-green
Connective tissue	pale red

Note

1. Blood smear or bone marrow smear should have uniform thickness to avoid affecting the staining effect.
2. After Giemsa staining in smear staining, do not remove the staining solution first or directly rinse the smear vigorously.
3. If the staining is too deep or too light, the staining time or working solution concentration should be adjusted.
4. In smear staining and tissue section staining, pH value has a certain impact on staining. Glass slides should be clean and free of acid-base contamination to avoid affecting the staining effect.
5. If the liquid level of the diluted dye has a metallic luster, it indicates that the dye has a dyeing effect, otherwise the dye may fail.
6. In tissue section staining, a large amount of 0.1-0.5% acetic acid should be used for rapid rinsing after staining to avoid contamination of the surface sediment and difficulty in washing off the sections.

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7. 0.5% acetic acid differentiation is commonly used for Giemsa tissue section staining, and can also be used for cell smears if necessary, but its concentration should be appropriately reduced; When the slices are differentiated with 0.5% acetic acid and turn pink, they can be terminated.
8. In Giemsa tissue section staining, dehydration with anhydrous ethanol should be rapid, otherwise the sections are prone to fading.
9. In smear staining and tissue section staining, if rapid results need to be obtained, Giemsa working solution can be prepared by mixing Giemsa Stain storage solution (10 ×): phosphate buffer solution=1:1. The mixture is thoroughly mixed to obtain rapid Giemsa staining working solution. Drop the staining solution onto the cell smear or tissue section, heat for staining, and add the staining solution again after 20-30 seconds, repeating 5-10 times. The rest of the steps are the same as above.
10. The staining solution can be reused, but cannot be reused multiple times. If there is sediment, it should be filtered before use.
11. Regaud fixative: prepared in a ratio of 3% potassium dichromate: formaldehyde=4:1, mixed well before use, and expired after 1-2 days.