# Giemsa Stain Solution (for Chromosomes)

# J1510432

**Storage** Room temperature.
**Shipping** Regular transportation.

## Introduction

Giemsa stain (also known as Gimsa stain) is a mixture of Azure II and Eosin. The staining principle and results of Giemsa staining are basically the same as those of Wright staining. Giemsa stain has a strong staining affinity for cytoplasm, and can well demonstrate the degree of cytoplasmic basophilia. In particular, it can clearly stain the azurophilic, eosinophilic and basophilic granules in blood and bone marrow cells. However, it stains cell nuclei too deeply, resulting in poor visualization of nuclear structures. Therefore, Giemsa stain solution is often used in combination with Wright stain solution. Many dyes have varying degrees of affinity for DNA and thus can be used for chromosome staining. For routine chromosome staining, satisfactory results can generally be obtained with Giemsa stain, Orcein stain, Feulgen stain, Carbol Fuchsin stain and other dyes. G-banding technology refers to treating chromosome slide specimens with trypsin, followed by staining with Giemsa stain. This process makes each chromosome display a certain number of horizontal bands with different widths and shades along its long axis, which are called banding patterns. Since the 22 types of human autosomes and the X and Y sex chromosomes each have distinct banding patterns, each chromosome can be clearly identified based on its banding pattern.

Aladdin Giemsa Stain Solution (for Chromosomes) consists of a 10× stock solution and phosphate buffer solution, which should be mixed at a ratio of 1:9 before use. It is mainly used for chromosome G-banding staining. The solution is prepared by grinding and mixing, with imported Giemsa stain and methanol as the main raw materials, plus a proprietary counterstain. It can produce clear cell staining results, and is commonly used for staining tissue sections, blood and cell smears, bacteria, chromosome banding, protozoan parasites, etc. This reagent is for research use only and is not suitable for clinical diagnosis or other purposes.

## Product Components and Storage Conditions:

| J1510432 | Component | 100mL | 500mL | Storage |
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| J1510432A | Giemsa Stain Stock Solution (10×) | 10mL | 50mL | RT |
| J1510432B | Phosphate Buffer Solution | 100mL | 500mL | RT |

## Self-prepared Materials:

1. PHA, colchicine, 0.075M potassium chloride solution, methanol-glacial acetic acid fixative

(methanol:glacial acetic acid = 3:1).

2. Constant temperature incubator, glass slides, microscopes.

## Operating Procedures (For Reference Only):

1. Prepare Giemsa Stain Working Solution: Mix Reagent (A) and Reagent (B) at a ratio of 1:9. Specifically, add 1 part of 10× Giemsa Stain Stock Solution to 9 parts of phosphate buffer solution and mix thoroughly to obtain the Giemsa Stain Working Solution. This working solution is ready-to-use, not suitable for long-term storage, and should be freshly prepared before use. If the staining effect is unsatisfactory, the mixing ratio can be adjusted accordingly.
2. Cell Culture: Collect 3–5ml of human peripheral blood and culture it in vitro for 3 days under conditions supplemented with PHA (general working concentration: approx. 60μg /ml) and serum. Add BrdU and/or colchicine (general working concentration: approx. 0.5 μg/ml), continue culturing for 30–60 min, then harvest the cells by centrifugation at 2000 g for 5 min and retain the cell pellet.
3. Hypotonic Treatment: Add 5–8ml of pre-warmed 0.075M potassium chloride solution (pre-heated to 37℃) to the cell pellet, incubate at 37℃ for 20–30 min for hypotonic treatment.
4. Cell Fixation: Add 1 ml of freshly prepared methanol-glacial acetic acid fixative to the pellet, mix gently, centrifuge at 2000 g for 5 min and retain the pellet. Add another 2–3 ml of methanol-glacial acetic acid fixative, mix gently, fix at room temperature for 15–20 min, centrifuge at 2000 g for 5 min and retain the pellet. Repeat this fixation step with another 2–3 ml of methanol-glacial acetic acid fixative and retain the final pellet.
5. Slide Dropping: Add a small amount of methanol-glacial acetic acid fixative to the cell pellet and mix gently to prepare a cell suspension. Drop 2–3 drops of the suspension onto pre-chilled glass slides. Blow away the water vapor fumes to promote cell rupture (alternatively, drop the cell suspension vertically from a height to induce cell rupture). Bake the slides at 70℃ for 2 hours.
6. Trypsin Digestion: Treat the baked slides with 0.025–0.05% trypsin solution for 30–90 min. The optimal digestion time should be determined empirically according to the concentration and batch of trypsin used.
7. Staining: Add sufficient Giemsa Stain Working Solution to cover the slide surface and stain at room temperature for 15–30 min.
8. Rinsing and Drying: Rinse the slide slowly from one end with tap water or distilled water to wash off excess staining solution, then air-dry the slide naturally.
9. Microscopic Examination: First, scan the entire slide under a low-power microscope to locate well-spread metaphase cells with chromosomes of moderate length. Then switch to an oil immersion lens to observe and identify the G-banding karyotype of the chromosomes.

## Precautions:

1. In the smear staining process, do not remove the stain first or rinse the smear vigorously directly after Giemsa staining.
2. If the staining is too dark or too light, adjust the staining time or the concentration of the working solution accordingly.
3. pH value has a certain impact on staining. Glass slides should be clean and free of acid-alkali contamination to avoid affecting the staining effect.
4. If the diluted stain shows a metallic luster on the liquid surface, it indicates that the stain is effective; otherwise, the stain may have expired.
5. The stain can be reused, but not for multiple times. If there is any sediment, filter the stain before use.