

Giemsa Stain Protocol (for visualization of blood microorganisms) Cat. No. ES915, ES915-16

Summary: Giemsa stain is a classical blood film stain for peripheral blood smear and bone marrow specimens. Erythrocytes stain pink, platelets show a light pale pink, lymphocyte and monocyte cytoplasm stain pale blue and leukocyte nuclear chromatin stains magenta.

Intended Use: This recommended protocol is applicable for the visualization of blood parasites. Giemsa Stain offers the flexibility of varying the intensity of the stain by adjusting staining times and the stain to buffer ratio. pH buffer selection will also affect your staining results. Suitable specimen is fresh whole anticoagulated blood prepared as a push smear. Giemsa Stain clearly defines in thick blood smears (6 - 20 layers thick) the various stages of the parasite presence of stippling (bright red dots) and fimbriations (ragged ends).

Materials Needed:

ES915	Giemsa Stain, 32oz
ES915-16	Giemsa Stain, 16oz
ES627, ES628	Methyl Alcohol (for fixation)
ES928	pH 7.2 Phosphate Buffer

Additional materials (for manual staining):

Plain microscope slides
Staining Coplin jars
Slide rack or forceps
DI or Laboratory grade water

Staining Jar Method:

1. Fix thin, air dried slides in Methyl Alcohol - approx. 1-3 minute.
(Very thick smears - do not fix, allow to air dry for 24 hours.)
2. Fill a Coplin jar with Giemsa stain prepared 1:10 with phosphate buffer, pH 7.2
3. Immerse the slide in stain-buffer mixture for 20-30 minutes.
4. Dip the stained smear into a second Coplin jar filled with phosphate buffer alone, 5-30 seconds for a thin smear, 3-5 minutes for a thick smear.
5. Rinse in DI water.
6. Allow slides to air dry and examine under oil immersion.

Staining Rack Method:

1. Set-up a staining rack over a sink. Prepare Giemsa stain by diluting 1:10 with pH 7.2 buffer.
2. Prepare slides as instructed. Allow smear to completely air dry followed by immediate fixation.
3. Flood the thick blood specimen with ~3 ml or an adequate volume of the stain solution, rock gently and allow to stain for 30 minutes.
4. Gently rinse the slide with the pH 7.2 buffer followed by a DI water rinse.
5. Allow slide to air dry. Examine using immersion oil.

NOTE: Staining intensity may be varied by increasing/decreasing the time in the Giemsa Stain/buffer mixture or by adjusting the stain to buffer ratio.

Storage Conditions:

Store at controlled room temperature (15-30°C). Keep bottle tightly closed when not in use. Do not use after expiration date specified on bottle label. Excessive precipitation in reagent is an indication of degradation.

Stained Specimen Results

If Plasmodium organisms are present, the cytoplasm will stain blue and the nuclear material will stain red to purple. Schuffner's stippling and other inclusions in the erythrocytes infected with Plasmodium spp. will stain red. Nuclear and cytoplasmic colors that are seen in the malarial parasites will also be seen in the trypanosomes and in the intracellular leishmania that are present.

<u>Specimen</u>	<u>Nucleus</u>	<u>Granules</u>	<u>Cytoplasm</u>	<u>Specimen</u>	<u>Nucleus</u>	<u>Granules</u>	<u>Cytoplasm</u>
Erythrocytes	Stippled Purple/blue	NA	blue/to pink	Leucocytes	purple/red stippling		blue/to pink
Leucocytes				Nongranular			
Granular				Monocytes	violet	NA	light blue
Neutrophils	purple	red-lilac	light pink	Lymphocytes	violet	NA	medium blue
Eosinophils	dark blue	red/orange	medium blue	Platelets	NA	purple	lilac
Basophils	dark blue	dark purple	light blue	Microorganisms	blue/violet	NA	NA

General Precautions:

Giemsa Stain Solution contains methyl alcohol and is a **Flammable Liquid. May be fatal or cause blindness if swallowed.** Do not breathe vapor. Avoid contact with eyes, skin, or clothing. Keep container closed. Use with adequate ventilation. Keep away from heat, sparks and open flame. Wash thoroughly after handling.

First Aid: If swallowed, call a physician immediately. In case of contact with eyes, immediately flush with water for 15 minutes and notify a physician. Wash exposed areas thoroughly after handling.

See SDS for complete product information.

All products are warranted to perform as described in their labeling and corresponding literature. All other warranties expressed or implied, including the warranty of merchantability and fitness for use are excluded. In no event shall Azer Scientific, Inc be liable for any indirect or consequential damages.

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