P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Fax (435) 755-0015 - www.scytek.com

Blue Feulgen DNA Ploidy Analysis Staining Kit

For Research Use Only.

Read entire specification sheet prior to using this product.

Introduction: ScyTek's Blue Feulgen Staining Kit is designed to identify deoxyribonucleic acid (DNA) in cell nuclei. After staining, the cells may be quantitatively evaluated for DNA content visually or using commercially available imaging systems. This kit is designed for cytological specimens prepared from cytospins, smears, cell imprints, disaggregated tissue, or whole tissue.

Principle: The ScyTek Blue Feulgen Staining Kit uses the Feulgen reaction to specifically and quantitatively stain DNA in cellular material. Cells are treated with a mild solution of hydrochloric acid which splits the purine (adenine and guanine) and pyrimidine (thymine and cytosine) bases from the sugar-phosphate groupings and exposes the aldehyde groups. Aldehyde groups uncovered in the hydrolysis react with the Feulgen stain to form a stable, colored compound. The amount of stain color developed is directly proportional to the amount of DNA present in the stained nuclei.

Contents:

Blue Feulgen Stain	2 x 500ml
Decolorizer	10 vials
Rinse Reagent	10 vials

Storage: The bottles of stain may be opened repeatedly and should be stored at 2-8º C. After conversion to a Schiff reagent, the stain should be used within 15 minutes. The vials of Decolorizer and Rinse are designed for single use and should be stored unopened at either 2-8ºC or 18-25ºC.

Precautions:

1. In both the staining procedure and rinse SO_2 is present. Procedure should be conducted in a well ventilated area, but keep SO_2 gas liberation to a minimum.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in staining area.
4. Avoid contact and/or inhalation of the various reagents.
5. Contain and clean-up any spills immediately.
6. Specimens, and materials exposed to them, should be considered biohazardous and handled as if capable of transmitting infection.
7. Follow all precautions to comply with federal, state, and local regulations for handling and disposal.

Additional Reagents and Supplies Required But Not Supplied:

1. Concentrated hydrochloric acid
2. 10% neutral buffered formalin
3. Xylene or Xylene substitute
4. Silanized glass slides
5. Coverslips
6. Mounting medium
7. Mixing containers
8. Coplin jars
9. Magnetic stir plate
10. Graduated cylinders

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11. Absolute ethanol
12. Deionized water
13. Distilled water
14. Parafilm
15. Forceps
16. Timer
17. Fume hood
18. Gloves, lab coat, goggles, and face shield
19. Cytocentrifuge

Specimen Preparation and Fixation: This kit may be used with cytological specimens prepared from whole tissue, disaggregated tissue, cell imprints, smears, or cytospins. Avoid using Bouin's fluid for fixation. Bouin's fluid hydrolyzes DNA and therefore affects the hydrolysis during staining. Staining can become negative with extended fixation in Bouin's fluid or other highly acidic fixatives.

Cell Imprints: The glass microscope slides should be clean and free from dust, oils, and lint. Holding tissue perpendicular to the surface of the slide, touch the slide lightly. Avoid smearing the sample. Air dry the slides, then fix for 60 minutes in 10% neutral buffered formalin. After formalin fixation, specimen slides should be washed in three changes of distilled water, 5 minutes each. After rinsing, air dry the slides for at least 60 minutes and store in a dust-free environment at room temperature (18-25ºC), until ready for staining.

Cytospins: Follow cytocentrifuge manufacturer's instructions. The glass microscope slides should be clean and free from dust, oils, and lint. Air dry the slides, then fix for 60 minutes in 10% neutral buffered formalin. After formalin fixation, specimen slides should be washed in three changes of distilled water, 5 minutes each. After rinsing, air dry the slides for at least 60 minutes and store in a dust-free environment at room temperature (18-25ºC), until ready for staining.

Tissue Deparaffinization:

1. Place slides in three changes of xylene for 5 minutes each.
2. Tap off excess xylene and place slides in two changes of absolute ethanol for 3 minutes each.
3. Tap off excess liquid and place slides in two changes of 95% ethanol for 3 minutes each.
4. Tap off excess liquid and place slides in 70% ethanol for 3 minutes.
5. Tap off excess liquid and place slides in two changes of distilled water for 3 minutes each.

Preparation of Reagents:

5N Hydrochloric Acid

Add 431ml of concentrated HCl (37%) to 569 ml of deionized water to make one liter of 5N HCl. Solution will become warm to the touch as acid is added to water. Stir gently for 60 minutes. Perform this operation in a fume hood, while wearing gloves, lab coat, goggles, and a face shield.

0.05N Hydrochloric Acid

Add 9 ml of 5N HCl to 891 ml of deionized water to make 900 ml of 0.05N HCl solution.

Blue Feulgen Stain

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Pour 100 ml of Blue Feulgen Stain solution in a beaker with a stir bar. Immediately prior to use, add the contents of one vial of Stain Decolorizer (white cap) to the Blue Feulgen Stain and stir. Stir until fully dissolved and solution is clear and yellowish. Solution should be used immediately.

Rinse Solution

Add the contents of one vial of Rinse Reagent (blue cap) to 400 ml of 0.05N HCl. Stir until fully dissolved and store in a tightly sealed container. For optimal results, make rinse solution fresh with each use. However, the solution can be stored in a tightly sealed container at 2-8ºC for up to one week.

Staining Procedure:

1. Hydrate fixed slides in distilled water for 5 minutes.
2. Hydrolyze slides in a coplin jar (plastic) containing 5N HCl for 60 minutes at room temperature (18-25ºC). Seal the jar completely with parafilm during incubation or cap tightly.
3. Place slides in distilled water for 2 minutes to remove excess acid.
4. Place slides in a coplin jar containing Blue Feulgen Stain (decolorized) for 60 minutes. Seal with parafilm or cap tightly. Occasionally a blue band may appear near the top of the staining solution. Prior to removing slides, the jar must be shaken to remove this band.
5. Rinse slides in three changes of distilled water for 2 minutes each. The slides can remain in the third change for up to 10 minutes while preparing for next step.
6. Place slides in three changes of Rinse Solution for 5 minutes each. Seal jars during rinse to reduce liberation of SO_2 gas into the laboratory.
7. Rinse slides in three changes of distilled water for 2 minutes each.
8. Dehydrate slides in 70% ethanol for 1 minute.
9. Dehydrate slides in two changes of 95% ethanol for 1 minute each.
10. Dehydrate slides in two changes of 100% ethanol for 1 minute each.
11. Clear slides in two changes of xylene for 1 minute each.
12. Coverslip in medium compatible with clearant used.

Expected Results:

The staining procedure will produce a blue color in the cell nuclei. The cytoplasm should be transparent with no staining. The intensity of blue color is directly proportional to the DNA content of the cell. Nucleoli within the cell nuclei should appear as light spaces, with dark edges, due to the nucleolar associated chromatin.
Stained slides remain stable for several years if stored protected from light.

Troubleshooting

Light to no staining:

1. Hydrolysis step may have been too long, removing DNA.
2. Incorrect fixative may have been used.
3. Staining time may need to be extended due to fixation issues.

Inconsistent Coloration:

1. Incorrect staining time.
2. Inadequate rinsing.
3. Cloudy mounting medium.
4. Strongly acidic mucous may occasionally stain blue/green.