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ab245878
Feulgen Stain Kit

For the quantitative evaluation of cellular DNA content for ploidy analysis

View Feulgen Stain Kit datasheet:

http://www.abcam.com/ab245878

[use http://www.abcam.cn/ab245878 for China, or http://www.abcam.co.jp/ab245878 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

The Feulgen Stain Kit is a histochemical reagent kit for quantifying the nuclear DNA content in cells. The kit can be used on a variety of specimen types from cytocentrifuge preparations to formalin fixed paraffin embedded tissue sections. A unique formulation eliminates dye precipitation and results in a cleaner stain than conventional Feulgen reagent-based kits. This stain kit is optimized for DNA Ploidy specimens that will be stained then scanned and viewed on image analysis systems. This kit is designed for cytological specimens prepared from cytospins, smears, cell imprints, disaggregated tissue, or whole tissue.

The amount of stain color developed is directly proportional to the amount of DNA present in the stained cells.

Staining Interpretation:

Nuclei:	Blue
Nucleoli:	Light spaces within the nuclei with dark edges
Cytoplasm:	Transparent with no staining

ΔNote: Strongly acidic mucous may occasionally stain blue/green.

Control Tissue:

Cytological specimens prepared from whole tissue, disaggregated tissue, cell imprints, smears, or cytospins.

Avoid Bouin's stain as it hydrolyses DNA.

2. Materials Supplied and Storage

Store kit at Room temperature immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Keep away from open flame and refer to the safety datasheet.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Blue Feulgen Stain	2 x 500 mL	4°C	4°C
Decolorizer	10 vials	RT	RT
Rinse Reagent	10 vials	RT	RT

3. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

ΔNote: 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 (18-25°C), 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 (18-25°C), until ready for staining.

5N HCl

- 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 HCl

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

Blue Feuglen Stain

- 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 2-8°C for up to one week.

Staining procedure

1.1 Hydrate fixed slides in distilled water for 5 minutes.

1.2 Hydrolyze slides in a coplin jar (plastic) containing 5N HCl for 60 minutes at room temperature (18-25°C). Seal the jar completely with parafilm during incubation or cap tightly.

1.3 Place slides in distilled water for 2 minutes to remove excess acid.

1.4 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.

1.5 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.

1.6 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.

1.7 Rinse slides in three changes of distilled water for 2 minutes each.

1.8 Dehydrate slides in 70% ethanol for 1 minute.

1.9 Dehydrate slides in two changes of 95% ethanol for 1 minute each.

1.10 Dehydrate slides in two changes of 100% ethanol for 1 minute each.

1.11 Clear slides in two changes of xylene for 1 minute each.

1.12 Coverslip in medium compatible with clearant used.

5. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 | +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829