BIOGNOST®

FEULGEN KIT

IVD *In vitro* diagnostic medical device CE

Five-reagent DNA staining kit according to Feulgen

INSTRUCTIONS FOR USE

REF Catalogue number: FE-100T (for 100 tests) FE-K-100 (6 x 100 ml)

Introduction

Feulgen reaction, first described by Robert Feulgen, is one of the most commonly used cytochemical methods for semiquantitative DNA determination in histological and cytological samples. It is very important to determine the exact amount and status of the DNA of the nucleus in order to make a diagnosis and treat malignant tumors. The key parameter in precise measuring of DNA is reproducibility of the Feulgen reaction. If the instructions for use are followed correctly, reproducibility is easily and reliably achieved by using reagents found in BioGnost's Feulgen kit. BioGnost's Feulgen kit contains additional contrasting reagent for cytoplasmic staining which enables easier and clearer observation of stained DNA. Using contrasting reagent is not necessary during the staining protocol, but it provides better contrast to the stained DNA of the section.

Product description

FEULGEN KIT – Kit for use in semiquantitative DNA determination.

The kit contains:	100 tests (FE-100T)	6 x 100 mL (FE-K-100)
HCL reagent, Feulgen	30 mL (HCLF-OT-30)	100 mL (HCLF-OT-100)
BioSchiff reagent	30 mL (BS-OT-30)	100 mL (BS-OT-100)
Sodium metabisulfite, solution for Feulgen	2x30 mL (NMF-OT-30)	2x100 mL (NMF-OT-100)
Sodium thiosulfate, 2% solution	30 mL (NT2-OT-30)	100 mL (NT2-OT-100)
Fast Green F.C.F. contrast reagent	30 mL (FGKR-OT-30)	100 mL (FGKR-OT-100)

Other sections and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 types of BioGnost's VitroGnost glass slides

Preparing the histological sections for staining

- Fixate the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue).
- Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

NOTE
Apply the reagent so it completely covers the section.

Sample staining procedure

a) using kit for 100 tests (FE-100T)

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Drip HCL reagent, Feulgen (≥5 drops)	40 min
6.	Rinse twice in distilled (demi) water	
7.	Add BioSchiff reagent (≥5 drops) *for a more intensive staining result, prolong the incubation up to 60 minutes	10 min
8.	Drain the section without rinsing. Remove the residual of the reagent from the section using filter paper	
9.	Add Sodium metabisulfite, solution for Feulgen (≥5 drops)	2 exchanges, 2 min each
10.	Drain the section without rinsing. Remove the residual of the reagent from the section using filter paper	
11.	Add Sodium thiosulfate, 2% solution	3 min
12.	Rinse the section in tap water	2 min
13.*	Add Fast Green F.C.F. contrasting reagent (≥5 drops)	10-15 seconds
14.*	Rinse with tap water	1 min
15.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
16.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
17.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

* Steps 13 and 14 include using contrasting reagent. If it is not necessary to counterstain the sections, skip these steps

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with a VitroGnost cover glass.

b) using five 100 mL reagents (FE-K-100)

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Immerse into HCL reagent, Feulgen	40 min
6.	Rinse twice in distilled (demi) water	
7.	Immerse in BioSchiff reagent *for a more intensive staining result, prolong the incubation up to 60 minutes	10 min
	Note: during staining cover the container with BioSchiff reagent in order to prevent sulfite evaporation	
8.	Drain the section without rinsing. Remove the residual of the reagent from the section using filter paper	
9.	Immerse into Sodium metabisulfite, solution for Feulgen	2 exchanges, 2 min each
	Note: during the treatment cover the container in order to prevent sulfite evaporation	
10.	Drain the section without rinsing. Remove the residual of the reagent from the section using filter paper	
11.	Immerse into Sodium thiosulfate, 2% solution	3 min
12.	Rinse the section in tap water	2 min
13.*	Add Fast Green F.C.F. contrasting reagent (≥5 drops)	10-15 s
14.*	Rinse with tap water	1 min
15.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
15.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
16.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

* Steps 13 and 14 include using contrasting reagent. If it is not necessary to counterstain the sections, skip these steps

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with a VitroGnost cover glass.

Result:

Nucleus - red-pink (magenta)
Cytoplasm and background - not stained if Fast Green F.C.F. contrasting reagent was not used; green if the contrasting reagent was used

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in reagents. Real staining protocol depends on personal requests and priorities.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory. In order to avoid an erroneous result, a positive and negative check is advised before application.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet.

Storing, stability and expiry date

Keep Feulgen kit in a tightly closed original package at room temperature. In order to ensure the quality and shelf life of the BioSchiff reagent, keep it at 2-8°C after first opening. Discard after it starts to assume color because of the SO_2 loss. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label. Valid BioSchiff reagent solution is colorless.

References

1. Kasten, F. H. (2003): Robert Feulgen and his histochemical reaction for DNA, *Biotechnic & Histochemistry,* 78 (1); p 45-49.
2. Millett, J. A. et al. (1982): Feulgen-hydrolysis profiles in cells exfoliated from the cervix uteri: a potential aid in the diagnosis of malignancy, *J. Clin. Pathol.* 35(3); p 345-349.
3. Pearse, A. G. E. (1972): Histochemistry: Theoretical and Applied, 3rd ed., London, Churchil Livingstone.
4. Schulte, E. et Wittekind, D. (1989): Standardization of the Feulgen-Schiff technique, *Histochemistry and Cell Biology,* 91 (4): p 321-331.

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Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
Refer to supplied instructions	Keep away from heat and sunlight	Valid until	LOT	Lot number		Manufacturer		
IVD For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile						