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Microscopy

DNA staining kit according to Feulgen

For the quantitative determination of DNA content in histological and cytological samples

IVD In Vitro Diagnostic Medical Device

CE

The Feulgen reaction is currently the method of the choice for the determination of DNA in histological and cytological specimens.
Since the importance of the DNA-content for the diagnosis and prognosis of malignant tumors was recognized, DNA image analysis is used more and more in these cases.

This “DNA staining kit according to Feulgen - For the quantitve determination of DNA content in histological and cytological samples” is used for human-medical cell diagnosis and serves the purpose of the histological and cytological investigation of sample material of human origin. It is a ready-to-use staining kit that when used together with other in vitro diagnostic products from our portfolio makes target structures (by fixing, embedding, staining, counterstaining, mounting) in histological and clinico-cytological specimen materials, for example histological sections of e.g. the kidney or the liver, evaluable for diagnostic purposes.

Principle

Feulgen’s reaction is based on the specific reaction between Schiff’s reagent and the 2-deoxyribose nucleic acids. The optimal degree of hydrolysis of DNA is reached only after the purine and pyrimidine bases have been cleaved off, resulting in the release of a maximum number of aldehyde groups. The quantity of DNA can be measured by light absorption. In contrast to this, RNA is unstable under the conditions of Feulgen’s reaction and cannot be detected.
The reproducibility of Feulgen’s reaction is of essential importance for the reliability of the DNA measurement. If the instructions for use are followed precisely, the closely coordinated reagents in this staining set enable the required degree of reproducibility.

Sample material

Cell culture monolayers, imprints (touch preparations), smears from fine needle aspiration biopsies (FNAB), smears from exfoliated cells, cytocentrifuged preparations from ascites, urine, liquor and other bodyfluids, just as tissue sections from formalin fixed, paraffin embedded tissue

Reagents

Cat. No. 1.07907.0001
DNA staining kit according to Feulgen
For the quantitve determination of DNA content in histological and cytological samples

Package components:

The staining kit contains 5 x 250-ml bottles

Reagent 1:	Hydrochloric acid 5 mol/l	2 x 250 ml
Reagent 2:	Schiff’s reagent	2 x 250 ml
Reagent 3:	Sodium disulfite solution (concentrate)	250 ml

Sample preparation

The sampling must be performed by qualified personnel.

For the fixation of the material different methods can be used.

Gynaecological smears

Spray fixation with M-FIX™	
Fixation in formaldehyde solution 4 % buffered (pH 6.9)	1 hour
Running tap water	10 minutes

Other cytological preparations

Air-dry	1 hour
Fixation in formaldehyde solution 4 % buffered (pH 6.9)	1 hour
Running tap water	10 minutes

Routinely formalin-fixed, paraffin-embedded tissue section

Deparaffinizing and rehydrating of the sections in the usual manner	
Running tap water	10 minutes

All samples must be treated using state-of-the-art technology.
All samples must be clearly labeled.
Suitable instruments must be used for taking samples and their preparation. Follow the manufacturer’s instructions for application / use.

Reagent preparation

The reagent 2 of the DNA staining kit according to Feulgen - For the quantitve determination of DNA content in histological and cytological samples is ready-to-use, dilution of the solution is not necessary and merely produces a deterioration of the staining result and their stability.

Sodium disulfite rinsing solution

Use only freshly prepared solutions.
For preparation of approx. 100 ml solution mix:

Reagent 3 (Sodium disulfite solution (concentrate))	5 ml
Distilled water	95 ml
Reagent 1 (Hydrochloric acid 5 mol/l)	1 ml

Procedure

The procedure is identical for all investigation materials.

Staining in the 60-ml Hellendahl cell

Deparaffinize histological slides in the conventional manner and rehydrate in a descending alcohol series.

The slides must be immersed and moved in the solutions, simple immersion alone yields inadequate staining results.

The slides should be allowed to drip off well after the individual staining steps, as a measure to avoid any unnecessary cross-contamination of solutions.

The stated times should be adhered to to guarantee an optimal staining result.

Slide with fixed smear or histological specimen	
Reagent 1 (Hydrochloric acid 5 mol/l) at 22 °C (± 0.5 °C)	50 min
Distilled water	2 min
Distilled water	2 min
Reagent 2 (Schiff’s reagent) at room temperature*	60 min
Sodium disulfite rinsing solution (freshly prepared)**	3 min
Sodium disulfite rinsing solution (freshly prepared)**	3 min
Distilled water	2 min
Distilled water	2 min
Ethanol 50 %	1 min
Ethanol 70 %	1 min
Ethanol 80 %	1 min
Ethanol 99 %	1 min
Xylene or Neo-Clear®	1 min
Mount the Neo-Clear®-wet slides with Neo-Mount® or the xylene-wet slides with e.g. Entellan® new and cover glass.	

* Due to the light-sensitivity of Reagent 2 (Schiff’s reagent), the staining should preferably be performed in the dark.

** The Sodium disulfite rinsing solution must be renewed after each staining schedule.

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, cytological and histological samples can be mounted with water-free mounting agents (e.g. Entellan® new, DPX new, or Neo-Mount®) and a cover glass and and can then be stored.

Measurement of the specimens

The covered specimen should be stored in the dark for 24 hours before the measurement. The refractive index of the mounting medium changes during this time and a reproduction of the measurement would be difficult.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Cell nuclei	red to violet
Cytoplasm	uncolored
Background	uncolored

Trouble-shooting

Weak staining intensity

- The temperature of reagent 1 (Hydrochloric acid 5 mol/l) was not kept exactly. The strict compliance of the hydrolysis temperature (22 °C ± 0.5 °C) is of essential importance for the reproducibility of the staining procedure.
- Reagent 2 (Schiff's reagent) is too old (see "Shelf-life").
- The temperature of Reagent 2 (Schiff's reagent) is too low. The reaction between the aldehyde groups of the DNA and the reagent is a chemical reaction and strictly dependent on temperature. Reagent 2 (Schiff's reagent) should be stored at room temperature.

Unspecific background staining

- Reagent 2 (Schiff's reagent) has reacted with an aldehyde group, which comes from the fixative. This can be avoided by careful washing of the specimens with the Sodium disulfite rinsing solution after the formaldehyde-fixation.
- Incomplete washing with the Sodium disulfite rinsing solution after the Feulgen reaction. The surplus Reagent 2 (Schiff's reagent) has not been removed completely. Careful washing with freshly prepared sodium disulfite rinsing solution is necessary.
- As a measure to obtain an indication of the intensity of the unspecific stain, a non-hydrolyzed specimen should be stained along as a negative control. As a result, this should be unstained (see "Diagnostics").

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
Follow the instructions of the manufacturer of the instrument for DNA-measurement by image analysis.
Follow the instructions for the histoprocessing and the instructions of the instrument and software company.
Remove surplus immersion oil before filing.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel.
Valid nomenclatures must be used.
Further tests must be selected and implemented according to recognized methods.

Suitable controls should be conducted with each application in order to avoid an incorrect result.
The absorbance values of the Feulgen-stained cell population must be compared to reference values of a normal, diploidic (2c) cell population. This makes it possible to evaluate the ploidy of a DNA distribution and to draw a correct diagnosis. When a cervical smear is analyzed, normal lymphocytes, intermediate cells, or granulocytes that are present on the slide can be used as a control.

Storage

Store the DNA staining kit according to Feulgen - For the quantitve determination of DNA content in histological and cytological samples at +15 °C to +25 °C.

Due to the light-sensitivity of reagent 2 (Schiff's reagent), the storage should preferably be performed in the dark.

Shelf-life

The DNA staining kit according to Feulgen - For the quantitve determination of DNA content in histological and cytological samples can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +15 °C to +25 °C.

The bottles must be kept tightly closed at all times.

The sodium disulfite rinsing solution must be renewed after each staining.

Reagent 2 (Schiff's reagent) must be colorless, because reddish colored reagent is useless (loss of SO_2) and should be discarded.

Capacity

The package is sufficient for 200 - 250 applications.

Reagent 1 (Hydrochloric acid 5 mol/l) is sufficient for approx. 500 applications.
Reagent 2 (Schiff's reagent) is sufficient for approx. 250 applications.
Reagent 3 (Sodium disulfite solution - concentrate) is sufficient for the preparation of approximately 5 l of Sodium sulfite rinsing solution.

Additional instructions

For professional use only.
In order to avoid errors, the application must be carried out by qualified personnel only.
National guidelines for work safety and quality assurance must be followed.
Microscopes equipped according to the standard must be used.
If necessary use a standard centrifuge suitable for medical diagnostic laboratory.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines.
Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at www.microscopy-products.com. Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing. Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

Auxiliary reagents

Cat. No.	100496	Formaldehyde solution 4%, buffered, pH 6.9 (approx. 10% Formalin solution) for histology	350 ml and 700 ml (in wide neck bottle), 5 l, 10 l, 10 l Tritripac®
Cat. No.	100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No.	103981	M-FIX™ spray fixative for cytodiagnosis	100 ml, 1 l
Cat. No.	103999	Formaldehyde solution min. 37% free from acid stabilized with about 10% methanol and calcium carbonate for histology	1 l, 2.5 l, 25 l
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100ml, 500 ml
Cat. No.	107164	Paraffin pastilles soldification point about 56-58°C for histology	10 kg (4 x 2.5 kg)
Cat. No.	107961	Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No.	108297	Xylene (isomeric mixture) for analysis EMSURE® ACS,ISO,Reag. Ph Eur	2.5 l, 4 l
Cat. No.	109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l
Cat. No.	111609	Histosec® pastilles soldification point 56-58°C embedding agent for histology	1 kg, 10 kg (4 x 2.5 kg), 25 kg
Cat. No.	115161	Histosec® pastilles (without DMSO) soldification point 56-58°C embedding agent for histology	10 kg (4 x 2.5 kg), 25 kg

Hazard classification

Cat. No. 1.07907.0001

Please observe the hazard classification printed on the label and the information given in the safety data sheet.
The safety data sheet is available on the website and on request.

Main components of the products

Cat. No. 1.07907.0001

Reagent 1

HCl 5 mol/l	187.5 g/l

Reagent 2

C.I. 42500	0.95 g/l
Na_2SO_3	13 g/l
1 l = 0.90 kg	

Reagent 3

$Na_2S_2O_5$	100 g/l

Other IVD products

Cat. No.	100380	ISOSLIDE® Iron Control Slides with reference tissue for the detection of free iron in histological tissue	25 tests
Cat. No.	105174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 2.5 l
Cat. No.	109033	Schiff's reagent for microscopy	500 ml, 2.5 l
Cat. No.	109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	109844	Eosin Y-solution 0.5% aqueous for microscopy	1 l, 2.5 l
Cat. No.	111674	Hemacolor® Rapid staining of blood smear staining kit for microscopy	1 set
Cat. No.	112327	LEUCOGNOST® Fixing Mixture for enzyme cytochemistry	500 ml


Consult instructions for use


Manufacturer

Catalog number

Batch code


Caution, consult accompanying documents


Use by YYYY-MM-DD


Temperature limitation

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