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Microscopy

Osmium(VIII) oxide

for microscopy

IVD In Vitro Diagnostic Medical Device



This “Osmium(VIII) oxide - for microscopy” is used for human-medical cell diagnosis and serves the purpose of the histological investigation of sample material of human origin. It is a fixative that when used together with other in vitro diagnostic products from our portfolio makes target structures (by fixing, embedding, staining, counterstaining, mounting) in histological specimen materials evaluable for diagnostic purposes.

Principle

Osmium tetroxide can be used to visualize unsaturated lipids. A part of the lipids is immediately blackened by the influence of osmic acid solution. The immediate black coloration results from the -C=C- double bond of the unsaturated fatty acid. The fatty acids that are made brownish at first can be blackened by treatment with 60 - 70 % alcohol.

For electron microscopy or semithin sectioning the glutardialdehyde-fixed specimens are post-fixed with osmium(VIII) oxide. Post-fixing with osmium(VIII) oxide enhances the contrast of the images of the lipids and thus of the lipid-rich organelles and structures.

Sample material

small tissue or organ specimens, only a few cubic millimeters in size and cryo sections

The fixed tissue can be further processed for semi-thin sections for light microscopy evaluation or for electron microscopy according to the relevant regulations.

Reagents

Cat. No. 124505
Osmium(VIII) oxide
for microscopy

100 mg, 500 mg, 1 g

Possibly also required:

Cat. No.	104239	Glutardialdehyde solution 25% for electron microscopy	250 ml, 1 l, 2.5 l
Cat. No.	112179	Glutardialdehyde solution 25% for electron microscopy, acc. to P.J. Anderson (purified and filled under nitrogen)	25 ml, 100 ml

Alternatively:

Cat. No.	109266	Osmic acid solution 2% for electron microscopy	5 ml (glass ampoule)
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Sample preparation

The sampling must be performed by qualified personnel.

Prior to fixing with osmium(VIII) oxide, the tissue specimens can be optionally fixed with 2.5 - 6.25 % glutardialdehyde solution (see corresponding instructions for use).

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

Phosphate buffer solution 0.1 mol/l, pH 7.2

For preparation of approx. 1000 ml solution mix:

Potassium dihydrogen phosphate	4.08 g
di-Sodium hydrogen phosphate anhydrous	12.46 g
Distilled water	1000 ml
dissolve	

Glutardialdehyde working solution

Dilute the 25 % stock solution with phosphate buffer solution 0.1 mol/l, pH 7.2 to a 2.5 - 6.25 % working solution.

Stock solution of osmium(VIII) oxide 2 %

Always use up the whole ampoule when preparing the osmic acid solution from osmium(VIII) oxide, always work in a fume cupboard as osmium fumes can irritate and damage the mucous membranes.

Clean the ampoule of Osmium(VIII) oxide - for microscopy, Cat. No. 124505, and remove the label, file the neck but not completely, and place in a suitable flask with a ground-glass stopper.

Crush the ampoule in the flask (e.g. with a screwdriver). Fill up with re-distilled water to give a 2 % stock solution of osmium(VIII) oxide.

Several hours are required for the dissolution of the crystals.

If the solution is colored black it is not suitable for use.

Use only re-distilled water.

Osmic acid solution 1 %, working solution

For preparation of the working solution mix:

Osmic acid solution 2%	1 part
Re-distilled water	1 part
mix	

Store the 1 % solution in the refrigerator at +2 °C to +8 °C.

If the solution is colored black it is not suitable for use.

Use only re-distilled water.

Procedure

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

Fixing without glutardialdehyde pretreatment

Slide with freshly prepared cryo sections		
Distilled water	rinse	
Osmic acid solution 1 %, working solution	fix	24 hours
Distilled water	wash thoroughly*	several hours
Ethanol 70 %	immerse	several hours
Distilled water	wash out*	
Mount with glycerol		

* changing the wash water frequently

Fixing with glutardialdehyde pretreatment

Immediately after taking the samples, fix the tissue specimens at 4 °C using 2.5 - 6.25 % glutardialdehyde solution*		at least 2 hours or overnight
Phosphate buffer solution 0.1 mol/l, pH 7.2	wash	
Osmic acid solution 1 %, working solution	post-fixing	24 hours
Distilled water	wash thoroughly**	several hours
Ethanol 70 %	immerse	several hours
Distilled water	wash out**	
Mount with glycerol		

* cooled glutardialdehyde solutions enhance the fixing result

** changing the wash water frequently

Result

Lipid inclusions	black
Background	yellow to brown

Technical notes

The microscope or electron microscope used should meet the requirements of a medical diagnostic laboratory.

Follow the manufacturer's instructions for use microtome and other devices.

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.

Storage

Store the Osmium(VIII) oxide - for microscopy at +15 °C to +25 °C.

Store the 1 % solution in the refrigerator at +2 °C to +8 °C.

Shelf-life

The Osmium(VIII) oxide - for microscopy - for electron microscopy can be used until the stated expiry date.

After opening the ampoule and preparation of the solution, this can be used up to the stated expiry date when stored at +2 °C to +8 °C.

The bottles must be kept tightly closed at all times.

Store the 1 % solution in the refrigerator at +2 °C to +8 °C.

If the solution is colored black it is not suitable for use.

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.

Always work in a fume cupboard as osmium fumes can irritate and damage the mucous membranes.

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.	100983	Ethanol absolute for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 5 l
Cat. No.	104095	Glycerol for fluorescence microscopy	250 ml
Cat. No.	104239	Glutardialdehyde solution 25% for electron microscopy	250 ml, 1 l, 2.5 l
Cat. No.	109266	Osmic acid solution 2% for electron microscopy	5 ml
Cat. No.	112179	Glutardialdehyde solution 25% for electron microscopy, acc. to P.J. Anderson (purified and filled under nitrogen)	25 ml, 100 ml

Safety classification

Cat. No. 124505

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 product

Cat. No. 124505

OsO₄ > 99.8 %

M = 254.20 g/mol

Other IVD products

Cat. No.	102419	Oil red O color solution for the detection of neutral lipids in cryo sections for microscopy	250 ml
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	115930	Toluidine blue O (C.I. 52040) for microscopy Certistain®	25 g

Literature

1. Romeis - Mikroskopische Technik, Editors: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg
2. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
3. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W. and Kiernan, J.A). Bios, 2002



Consult instructions
for use



Manufacturer



Catalog number



Batch code



Caution, consult
accompanying documents



Use by
YYYY-MM-DD



Temperature
limitation

Status: 2017-08-16

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