



“Eosin Y solution 0.2% alcoholic” is used in human medicine cell diagnostics and serves for the histological and clinical-cytological examination of human samples. It is a ready-to-use dye solution that makes target structures in histological and clinical-cytological specimen, e.g. histological slices of, for example, kidney, muscle, heart, lung, evaluable for diagnostics (by means of fixation, embedding, staining, counter-staining, mounting).

Hematoxylin-eosin staining (H&E) is the most commonly used staining method for histological material. “Eosin Y solution 0.2% alcoholic” is used in the hematoxylin-eosin staining (H&E), which is commonly applied in histology.

Staining mechanism:

The first step of the H&E-staining mechanism is a coulomb interaction of the positively charged nuclear stain (hematoxylin) with the negatively charged phosphate groups of the nucleic acids in the cell nucleus. The nuclei appear in dark blue to dark violet. The second step is the counter-staining with an anionic xanthene dye (eosin G, eosin B or erythrosine B). It binds to the positively charged plasma proteins. Cytoplasm and intercellular substances will be stained pink to red, erythrocytes appear in yellow-orange or red-orange.

A differentiation is made between the progressive hematoxylin staining, which entails staining until the endpoint and subsequent bluing and fixation in tap water, and the regressive method, which will be described below. Here, overstaining with hematoxylin occurs, the excessive dye is removed in acidic differentiating steps and bluing and fixation of the staining with tap water takes place. With the regressive staining, the nucleus structures appear more differentiated and are more clearly visible.

Tissue used:

Slices of formalin-fixed, paraffin-embedded tissue (3-4 µm thick paraffin slices) or frozen sections serve as the source material as well as clinical material from cytology, e.g. urine sediment, sputum, smears of fine-needle aspiration cytology (FNAC), flushing liquids, imprints, effusions, may be used.

Sample preparation:

Sample collection must be performed by qualified personnel.

All samples must be handled according to the state of the art. All samples must be labelled unambiguously. Suitable instruments must be used for sample collection and preparation; the

manufacturer's instructions for their application/use must be followed.

Slices must be dewaxed and rehydrated in typical manner.

Reagent preparation:

The “Eosin Y solution 0.2% alcoholic” used for staining is ready-to-use; diluting of the solution is not necessary and would minimize the staining result and its shelf-life. It is recommended to filter the solution prior to use.

To intensify the eosin staining, add, for example, 1.0 ml glacial acetic acid to 500 working solution.

The acidified working solution is sufficient for approx. 750 specimens, but it should be renewed after 14 days at the latest.

When using “Eosin Y solution 0.2% alcoholic”, a decreasing alcohol concentration has to be used in performing each staining (starting with ethanol 96 % and only 10 seconds application time).

Performing the H&E-staining

Regressive staining of paraffin slices

Staining in the staining cuvette

Dewax histological specimens in typical manner and rehydrate with decreasing alcohol concentration. The slides should be well drained after each staining step to avoid unnecessary carryover of solutions. To achieve an optimal staining result, adhere to the times indicated.

Slide with paraffine slice	
Distilled water	1 min.
“Mayer's hamalum solution” (art. no.: 6.00.05.0001)	3 min.
Hydrochloric acid 0.1% aqueous	2 sec.
Running tap water	3 - 5 min.
“Eosin Y solution 0.2% alcoholic” (art. no.: 6.00.05.0003)	3 - 5 min.
Running tap water	30 sec.
Ethanol 96%	10 sec.
Ethanol 96%	10 sec.
Ethanol 100%	1 min.
Ethanol 100%	1 min.
Xylene	5 min.
Xylene	5 min.
Mounting with EUKITT® (art. no.: 6.00.01.0001), EUKITT® neo (art. no.: 6.00.01.0003) or EUKITT® UV (art. no.: 6.00.01.0005).	
With EUKITT® UV or EUKITT® neo, the two xylene steps can be omitted.	

After dehydration (increasing alcohol concentration), histological specimens may clarify with xylene and be mounted and stored with non-aqueous mounting medium (e.g. EUKITT®, EUKITT® neo or EUKITT® UV) and coverslip.

For analysis of stained specimens with microscopic enlargement >40x, the use of immersion oil is recommended.

Evaluation:

Nuclei	dark blue to dark violet
Cytoplasm, intercellular substances	pink to red
Erythrocytes	yellow to orange

If a weak staining of the cytoplasm and the connective tissue structures occurs in the eosin staining, the use of a working solution acidified with glacial acetic acid is recommended.

Technical information:

The microscope used should comply with the requirements of a medical-diagnostic lab. If histo-processors or stainers are used, the hardware and software manufacturers' instructions are to be followed. Remove excessive immersion oil prior to archiving.

Diagnostics:

Only authorized and trained personnel may give diagnoses. Valid nomenclature is to be used. Follow-up tests are to be chosen and performed according to recognized methods.

Storage:

“Eosin Y solution 0.2% alcoholic” must be stored at +15 °C to +25 °C (+59 °F to +77 °F).

Storage temperatures below +15 °C (+59 °F) may cause dye precipitation in the solution. In that case, the dye solution should be put into a water bath at approximately 60 °C (140 °F) for 2-3 hours and be filtrated prior to use.



Warning:

Please read all information carefully before use.



Biohazard warning:

Use appropriate personal protective equipment when handling potentially infectious sample material.



Do not use if packaging is damaged:

If the packaging is damaged, this may lead to leakage of “Eosin Y solution 0.2% alcoholic”. In general, be aware of the dangers of wetting and take appropriate safety measures to prevent this (e.g. wearing gloves).



Use by:

“Eosin Y solution 0.2% alcoholic” may be used until the stated expiry date.

After first opening, store bottle at +15 °C to +25 °C (+59 °F to +77 °F) and use until expiry date.

Always keep bottles properly closed.

Directions for Use:

“Eosin Y solution 0.2% alcoholic” is ready-to-use and may be applied without further preparation steps.



Follow the usage instructions:

“Eosin Y solution 0.2% alcoholic” should be used in accordance with the usage instructions provided by relevant system and/or reagent supplier, or according to your own tried-and-tested methods.

National guidelines for occupational safety and quality assurance are to be observed. Microscopes equipped according to the standard are to be used.



Warning:

For professional use only!

To avoid errors, application must be performed by trained personnel.

Disposal instructions:

Dispose of packaging according to existing disposal regulations. Used solutions and solutions with expired shelf-life must be disposed of as hazardous waste, the local disposal regulations are to be observed. 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 within the EU.

“Eosin Y solution 0.2% alcoholic”:

Art. no.: 6.00.05.0003.07.04.01 1000 ml

Manufacturer:



ORSATec GmbH
Max-Fischer-Straße 11
86399 Bobingen
Germany



Tel. +49 761 81077
Email: info@orsatec.de
<http://www.eukitt.de>

“Eosin Y solution 0.2% alcoholic”:

Art. no.: 6.00.05.0003.07.04.01

Hazard and precautionary statements:

Flammable liquid and vapour (H226).

Keep away from heat/sparks/open flames/hot surfaces. No smoking (P210). Use explosion-proof electrical/ventilating/lighting/equipment (P241). Wear protective gloves/protective clothing/eye protection/face protection (P280). Ground/bond container and receiving equipment (P240). Keep container tightly closed (P233). Use only non-sparking tools (P242). Take precautionary measures against static discharge (P243). IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower (P303 + P361 + P353). Store in a well-ventilated place. Keep cool (P403 + P235). Dispose of contents/container in accordance with local/regional/national/international regulation (P501).