**Instruction manual**

**Eosin Y solution 0.2% alcoholic**

“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 blueing 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 blueing 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:**
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 histoprocessors 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.
“Eosin Y solution 0.2% alcoholic”:
Art. no.: 6.00.05.0003.07.04.01

Hazard and precautionary statements:
Flammable liquid and vapour (H226).