



3E-096

Hayem reagent

In-vitro diagnostic agent

Description

The product 3E-096 is a ready-to-use solution for professional users in haematology. The product comes in 3 different pack sizes: 3E-096.00100 (100ml bottle), 3E-096.00250 (250ml bottle), 3E-096.01000 (1l bottle).

Main components

Mercury chloride (CAS 7487-94-7))	6.25g/l
Sodium chloride (CAS 7647-14-5))	12.5g/l
Sodium sulphate (CAS 7757-82-6)	62.5g/l

Purpose

The dye "Hayem's reagent" is used for cell diagnostics. It is a ready-to-use solution for manual red blood cell counting.

Sample material and sample preparation

Sampling may only be carried out by qualified personnel. All samples must be processed with state-of-the-art technology. All samples must be clearly labelled.

Sample material: Venous blood, EDTA whole blood

Test principle

Staining with "Hayem's reagent" is considered a routine method for counting erythrocytes. The reagent prevents erythrocyte agglutination. Sodium sulphate and sodium chloride contribute to the osmolality of the solution and protect the erythrocytes from haemolysis. Mercury chloride serves as a preservative and prevents the blood from clotting, so that a precise count of the cells is possible.

The basic prerequisite is a targeted dilution and specimen of the blood sample. The desired cell type is counted in a defined volume and the cell number in one microlitre of blood is calculated.

Staining

Additional materials: Counting chamber according to Neubauer, Bürker or Fuchs-Rosenthal, calibrated erythrocyte pipette.

The blood is aspirated in the calibrated red cell mixing pipette up to the 0.5 mark (up to 1.0 in the case of severe anaemia) and then Hayem's reagent is aspirated up to the 101 mark. The blood must not contain any air bubbles. Native blood may clot, so work quickly. This results in a dilution of 1:200 (or 1:100). Then mix the mixture well in the pipette for 1-2 minutes. Discard the first 3 drops from the pipette. Then pour the mixture into a counting chamber prepared by a cover slip. To check, also fill the opposite side of the counting chamber. If air bubbles are visible or if liquid has run over the edges into the grooves, the chamber must be cleaned and refilled.



Counting under the microscope

With the help of the counting nets, 80 smallest squares around the centre of the so-called Thoma net are counted with the 40x objective, whereby attention should be paid to an even distribution of the erythrocytes. Only those erythrocytes that are clearly located in the group square (corresponds to 16 basic squares and boundary lines, e.g. top and left) are taken into account in the counting.

Result

Calculation of the erythrocyte count

$$\begin{aligned}
 \text{Erythrocyte count} &= X \times V \times 4000 / 80 \\
 &= X \times 10000 \text{ [cells/ } \mu\text{l]} \\
 &\quad (V=200) \\
 &= X \times 5000 \text{ [cells/ } \mu\text{l]} \\
 &\quad (V=100) \\
 &= X \times 0.01 \text{ [cells/ pl]} \\
 &\quad (V=100)
 \end{aligned}$$

V = dilution factor (200 or 100)

X = the number of erythrocytes found in 80 smallest squares

Normal range

	Erythrocytes/ μl	Erythrocytes/ pl
Women	4.0 - 5.5, $\times 10^6$	4.0 - 5.5
Men	4.0 – 6.0 $\times 10^6$	4.0 - 6.0
Children	4.0 – 5.5 $\times 10^6$	4.0 - 5.5
Newborns	4.0 – 7.0 $\times 10^6$	4.0 – 7.0

Precautionary measures

When removing the product, care must be taken to avoid contamination of the storage vessel. Once the solution has been removed, it must not be returned to the canister. If turbidity or solids appear, discard the product. The product is intended for single use and must not be reused.

Storage and shelf life

Store the unopened containers in a dry place at 15 to 25 °C, avoiding direct sunlight.

The shelf life is 2 years. See also the best-before date (BBD) on the label. Once the containers have been opened, the shelf life corresponds to the best-before date, as long as the storage conditions are observed and the solution is handled properly.

Safety notice

If any serious incidents occur in connection with the product, please report them to the manufacturer and the national authority.



Literature

Romeis, Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2010, Springer Spektrum, 18th edition

<https://www.phys.szote.u-szeged.hu/edu/germana/praktikumsbucht1.pdf>