2C-327
Gram Staining Set

IVD

In Vitro Diagnostic

Description

The product 2C-327 is a ready-to-use staining set for Gram staining for the professional user for application in human-medical cell diagnostics and contains 5 different solutions. The product is supplied as a set of 5 bottles of 500 ml each: 2C-327.00001.

Main components

Solution 1	
Gentian violet (C.I. 42535)	3.6 g/l
Phenol (CAS 108-95-2)	8.0 g/l
Solution 2	
Iodine (I_2)	3.4 g/l
Potassium iodide (KI)	6.8 g/l
Solutions 3 and 4	
Ethanol (CAS 64-17-5)	50%
Acetone (CAS 67-64-1)	50%
Solution 5	
Safranin O (C.I. 50240)	9.5 g/l
Ethanol (CAS 64-17-5)	158 g/l

Intended use

The "Gram Staining Set" is used for cell diagnostics for the examination of microbacteria of human origin. It is a ready-to-use staining set for the professional user. It can be used for staining microscopic preparations in microbiology to distinguish bacterial targets. Gram staining allows rapid differentiation of bacteria into Gram-positive and Gram-negative.

Sample material and sample preparation

Samples may only be taken by qualified personnel. All samples must be handled according to the state of the art. All samples must be clearly labelled.

Sample material: smears of bacteriological material, after air drying and heat fixation, such as sputum, fine needle aspiration biopsy (FNAB) smears, irrigation fluids, imprints, effusions, pus, exudates, liquid and solid enrichment cultures.

Test principle

The cell walls of the bacteria determine the staining behaviour of the bacteria. After staining the bacteria with crystal violet, an aniline stain, a stain-iodine complex, is formed after the addition of the iodine solution (Lugol's solution).

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The cell walls of Gram-positive bacteria are based on a multilayered mural scaffold, which prevents the stain-iodine complex from being washed out during the decolourisation step. The bacteria remain blue-violet in colour. Gram-negative bacteria, on the other hand, have a cell wall consisting of a single-layer mural scaffold, so that the stain is released again during decolourisation. Counterstaining with saffronine solution stains the Gram-negative bacteria pink to red.

Staining

The staining set for Gram staining is ready to use, and only dilution of the solutions with distilled water is necessary for staining in the cuvette for reagent 1 (Grams crystal violet) (ratio 1:3).
For staining, the samples must be immersed in the solution and moved. After the respective staining steps, the samples must be properly drip cleaned.
First, the samples are stained using crystal violet solution (solution 1) and then washed under running tap water. For stabilisation, the samples are placed in Lugol's solution (solution 2). After washing under running tap water, the samples are decolourised with the third and fourth solutions and washed again. Counterstaining is then carried out using saffronine solution (solution 5). After rinsing under running tap water, the samples are air-dried (e.g. overnight or at 50°C in a drying cabinet).
To ensure the differentiability of the target structures, suitable control preparations must be maintained with the staining.

The standard staining protocols known from the literature must be used.
Staining may only be carried out by qualified personnel.

Result

Gram-positive microorganisms	blue-violet
Gram-negative microorganisms	pink to red

Precautions

When withdrawing 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

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. After opening the containers, the shelf life corresponds to the best-before date, provided that 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 regulator.

Bibliography

Romeis, Microscopy Technique, Editors: Maria Mulisch, Ulrich Welsch, 2010, Springer Spektrum, 18th edition

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