

Insert for Kit 98011-12-14

ESBL Confirm Kits

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FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for Beta-lactamase identification

MANUFACTURE: ROSCO, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark.

INTENDED USE: Tablets are used for *in vitro* identification of microbial resistance mechanisms by the agar tablet/disc diffusion method, in order to confirm the mechanism by which the organism has gained resistance to specific antimicrobial agents.

INTENDED USERS: To be used only by professionals, qualified laboratory personnel and people trained to work with microbes and disc diffusion testing.

TEST PRINCIPLE: ROSCO has developed a series of confirmatory (and screening) kits for the routine detection of ESBL producing isolates among the species Enterobacteriaceae. The choice of test kit depends on the isolate to be tested, and whether or not it produces chromosomal AmpC. Therefore, the isolate should be identified beforehand, and assigned in to one of the two following groups:

Group 1:

E. coli, Klebsiella spp., P. mirabilis, Salmonella spp. and Shigella spp.

- Recommendation CLSI/EUCAST: Use of Cefotaxime and Ceftazidime and their combination with Clavulanate.
- If the isolate is, Cefoxitin resistant (zone < 19mm) also test for Cefepime and Cefepime+ Clavulanate as well.

Group 2:

Enterobacter spp., Serratia spp., Providencia spp., C. freundii, Morg. morganii and Hafnia alvei (all producing chromosomal AmpC)

- Recommendation EUCAST: Use Cefepime and Cefepime + Clavulanate

Use of the Total ESBL Confirm Kit includes detection of all Enterobacteriaceae, including chromosomal AmpC producers.

Use of the ESBL Confirm (acc. to CLSI/EUCAST) Kit includes detection of isolates in group 1 and use of the ESBL Confirm (Chrom. AmpC) Kit includes detection of isolates belonging to group 2. Furthermore, the ESBL Screen Kit allows for the routine screening of ESBL producing bacteria in all Enterobacteriaceae.

Generally, isolates showing the following zones of inhibition should be suspected of producing ESBL's:

- <= 21 mm with Cefotaxime 5 ug and/or <= 22 mm with Ceftazidime 10 ug.
- <= 27 mm with Cefotaxime 30 ug and/or <= 22 mm with Ceftazidime 30 ug.
- and/or <= 17 mm with Cefpodoxime 10 ug.

DETAILED INSTRUCTIONS:	<p>ROSCO's detailed <i>Instruction for Use for Detection of resistance mechanisms</i> should be available in laboratories working with ROSCO's Diagnostic products. Latest version of Instruction for Use can be seen in and/or printed out from ROSCO's website www.rosco.dk</p> <p><i>User's Guide</i> can be obtained free of charge from your local distributor on request, or from ROSCO:</p> <p>E-mail: info@rosco.dk</p> <p>Phone: +45 43 99 33 77</p>
CONTENT AND FORMULATION:	<ol style="list-style-type: none"> <p>1. Total ESBL Confirm Kit (98014) Comprises one cartridge (50 tests) each of the following: Cefotaxime 30 µg Cefotaxime 30 µg + Clavulanate Ceftazidime 30 µg Ceftazidime 30 µg + Clavulanate Cefepime 30 µg Cefepime 30 µg + Clavulanate Testing of any kind of Enterobacteriaceae, including chromosomal AmpC producers.</p> <p>2. ESBL Confirm (acc. to CLSI/EUCAST) Kit (98011) Comprises one cartridge (50 tests) each of the following Cefotaxime 30 µg Cefotaxime 30 µg + Clavulanate Ceftazidime 30 µg Ceftazidime 30 µg + Clavulanate Testing of Enterobacteriaceae Group 1 (without chromosomal AmpC) according to CLSI/EUCAST.</p> <p>3. ESBL Confirm (Chrom. AmpC) Kit (98012) It comprises two cartridges (100 tests) each of the following: Cefepime 30 µg Cefepime 30 µg + Clavulanate Testing of Enterobacteriaceae Group 2 (possessing chromosomal AmpC) according to EUCAST.</p>
STORAGE/HANDLING:	<p>Store at 2-8 °C until the expiration date shown on the product label. Cartridges should be closed during storage. Always seal the cartridges with the original green lid and never place the dispenser in the refrigerator.</p> <p>Allow the cartridges to acclimatize at room temperature (30-60 min) before removing the lid. Cartridges may open and close several times during use, without affecting tablets' shelf-life. The long shelf-life is due to the use of crystalline substances.</p>
PRECAUTIONS:	<p>For <i>in vitro</i> diagnostic use only. Safety precautions should be taken and aseptic techniques should be used when working with potential biohazards. To be used only by adequately trained and qualified laboratory personnel. Sterilize all biohazard waste before disposal. Refer to Product Safety Data Sheet.</p>
REQUIRED BUT NOT PROVIDED MATERIALS:	<p>Standard microbial equipment such as loops, culture media, incubator etc. and biochemical reagents.</p>

PROCEDURE:

1. Using a fresh, pure culture prepare a suspension of the organism to be tested equivalent to McFarland 0.5.
2. Using a sterile swap or Drigalski spatula spread the suspension uniformly over the entire area of a Mueller Hinton susceptibility agar plate.
3. Using a single tablet dispenser, place one of each tablet on the inoculated agar plate, ensuring sufficient space between individual tablets to allow for proper measurement of inhibition zones. Notice that more than one Confirm Kit can be tested on the same plate.
4. Incubate at $35\pm 1^\circ\text{C}$ for 18 ± 2 hours (overnight).
5. Measure and record the diameter of the inhibition zones. No zone around a tablet corresponds to a 9 mm inhibition zone.

INTERPRETATION OF RESULTS:

The results are interpreted by comparing the inhibition zones around the different diffusion discs/tablets. Compare the zone of inhibition around the Cephalosporin + Clavulanate combination with the zone around the Cephalosporin alone. If the inhibition zone diameter around the combination disc is ≥ 5 mm larger than the one around the single cephalosporin disc/tablet the isolate is showing ESBL production.

Frøding et al (1) using the EUCAST disc diffusion test with Neo-Sensitabs (Rosco ESBL detection kits) evaluated the accuracy of reading EUCAST disc(tablet) diffusion after 6 hours' incubation. They concluded that **inhibition zone reading at 6 hours**, is an accurate method for detecting ESBLs in Enterobacteriaceae.

QUALITY CONTROL:

Although ROSCO produces the most stable diffusion discs (tablets) it is necessary to perform regular quality control. This should be done with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained using the combination tablets plus the cephalosporin alone disc against the negative control (i.e. E. coli ATCC 25922), should be within 3 mm. Any greater difference indicates that the product has lost activity and should not be used.

As positive Q.C. strains the following may be used:

Klebsiella pneumoniae ATCC 700603, ESBL positive, (except cefepime)

Enterobacter cloacae NCTC 13464, ESBL positive (use Cefepime and FEP+C)

REFERENCES:

1. Frøding I et al: Rapid EUCAST disc diffusion testing of MDR E. coli and K. pneumoniae: inhibition zones for ESBL can reliably read after 6 hours of incubation. J Antimicrob Chemother Dec 20, 2016 (ahead of print).