

Insert for Kit 98007

AmpC Confirm ID Kit

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LANGUAGE: English

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: This Kit consists of four cartridges of disc diffusion tablets: one cartridge of tablets with Cefotaxime, one with Ceftazidime and two cartridges of the cephalosporins combined with Cloxacillin (AmpC inhibitor). If an organism is suspected of AmpC activity, it can be shown by a difference in the inhibition zone of the cephalosporin(s) alone and in combination with the inhibitor.

DETAILED INSTRUCTIONS: ROSCO's detailed *Instruction for Use for Detection of resistance mechanisms* 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 *User's Guide* can be obtained free of charge from your local distributor on request, or from ROSCO:
E-mail: info@rosco.dk
Phone: +45 43 99 33 77

CONTENT AND FORMULATION: 4 cartridges, formulated for maximum stability, each containing approximately 50 tablets:

- 1 Cefotaxime 30 µg, coded CTX30
- 2 Cefotaxime 30 µg + Cloxacillin, coded CTXCX
- 3 Ceftazidime 30 µg, coded CAZ30
- 4 Ceftazidime 30 µg + Cloxacillin, coded CAZCX

STORAGE/HANDLING: 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.

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.

- PRECAUTIONS:** For *in vitro* 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.
- REQUIRED BUT NOT PROVIDED MATERIALS:** Standard microbial equipment such as loops, culture media, incubator etc. and biochemical reagents.
- 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 zone. No zone around a tablet corresponds to a 9 mm inhibition zone.
- INTERPRETATION OF RESULTS:** The results are interpreted by comparing the inhibition zones of the different tablets.
- 1) Compare the zone of inhibition of Cefotaxime 30 μg and Ceftazidime 30 μg tablet to the zones of inhibition of Cefotaxime 30 μg + Cloxacillin and Ceftazidime 30 μg + Cloxacillin tablets, respectively. If these zones are within 3mm of each other, record the organism as not expressing AmpC activity.
 - 2) Measure the inhibition zones around Cefotaxime 30 μg + Cloxacillin (CTXCX) and Ceftazidime 30 μg + Cloxacillin (CAZCX), and compare with the respective zones around Cefotaxime 30 μg (CTX30) and Ceftazidime 30 μg (CAZ30). If one or both of the combination discs show zones $\geq 5\text{mm}$ than the single discs, the organism is demonstrating AmpC activity.
 - 3) Use Table 1 to assist in the interpretation
- 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 tablet 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. stains the following may be used:
Enterobacter cloacae NCTC 13406, AmpC positive
Enterobacter cloacae ATCC BAA – 1143, Amp C positive
E. coli ATCC 25922 is a negative Q. C. stain

Table 1.

		Cefotaxime 30 µg CTX30	Ceftazidime 30 µg CAZ30
AmpC	CTXCX	≥ 5mm	-
AmpC	CAZCX	-	≥ 5mm

No AmpC activity: All zones in the combinations within 2 mm of the corresponding single agents.

Note: “-” means that the difference is irrelevant (i.e. CTXCX should not be compared with CAZ30 for the detection of AmpC)

REFERENCES:

- 1) Ascelijn RE et al: Detection and occurrence of plasmid mediated AmpC in highly resistant gram negative rods. PLOSONE, 9, e91396, 2014.