

## Insert for Kit 98022

### Rapid ESBL Screen Kit

**REVISION:** DBV0041G

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**LANGUAGE:** English

FOR IN VITRO DIAGNOSTIC USE ONLY

- PRODUCT GROUP:** Kits for detection of resistance mechanisms.
- MANUFACTURE:** ROSCO Diagnostica ApS, Stensmosevej 24 , DK-2640 Albertslund.
- INTENDED USE:** Tablets are used for in vitro screening of ESBL producing bacteria. The method is valid for Enterobacterales.
- INTENDED USERS:** To be used only by professionals, qualified laboratory personnel and people trained to work with microbes and disc diffusion testing.
- TEST PRINCIPLE:** Potential ESBL-producing bacteria are currently screened by the means of susceptibility testing of cephalosporins (Ceftazidime, Cefotaxime, Cefpodoxime). Reduced inhibition zones around these cephalosporins are used to indicate possible ESBL production. A rapid method is based on the identification of the hydrolysis of the beta-lactam ring of a cephalosporin in the presence of an indicator. Utilizing this principle ROSCO has developed two new Diatabs; Cefotaxime + Indicator (ESBL) and Cefotaxime + Tazobactam + Indicator (ESBL). The test is performed quickly and the reading of the results is ready within **15 minutes to one hour**, from the time the reaction is started. Thus, applying this kit, in the routine screening of ESBLs, saves time and effort in the laboratory.  
The idea is to help the laboratory to perform their own ESBL screening.  
The antimicrobial stability in the Rosco Diatabs is at least 3 years.
- DETAILED INSTRUCTIONS:** ROSCO's detailed Instruction for Use of DIATABS should be available in each laboratory working with ROSCO's Diagnostic products.  
The latest edition of Instruction for Use is available at ROSCO's website [www.rosco-diagnostica.com](http://www.rosco-diagnostica.com) More detailed information can be found in ROSCO's User's Guide for Detection of resistance mechanisms in English.  
Instructions for Use and User's Guide can be obtained free of charge from your local distributor on request, or from ROSCO:  
E-mail: [info@rosco-diagnostica...dk](mailto:info@rosco-diagnostica...dk)  
Phone: +45 93 40 65 65
- CONTENT AND FORMULATION:** One vial with 6 mm tablets; Cefotaxime + Indicator (ESBL) Diatabs, formulated for maximum stability, each containing 50 tablets equivalent to a total of 50 tests.  
One vial with 6 mm. tablets: Cefotaxime + Tazobactam + Indicator (ESBL) Diatabs, 50 tablets.
- 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:**

Saponin 5% solution. Buffer B-PER II, Bacterial Protein Extraction Reagent. Standard microbial equipment such as loops, culture media, incubator etc. and biochemical reagents.

**PROCEDURE:**

Use always fresh isolates. Otherwise, inoculate/incubate the isolate 2 times before testing.

Colonies should be taken from the following media: Mueller-Hinton Agar, Blood agar or TSA agar.

Do not use colonies from selective agars (Drigalski, Mc Conkey)

Add one 10 ul loop of the strain to be tested (recovered from antibiogram) to a mixture of 150 ul 0.9 % NaCl solution + 50 µl Tris-HCL 20 mmol/l lysis buffer (B-PER II; Bacterial Protein Extraction Reagent, Thermo Scientific). Standard Bug Buster Reagent Tris buffered pH 7.5 (NOVAGEN) can also be used.

Vortex the suspension for one minute and maintain at room temperature for 30 min. Add 50 µl of the bacteria suspension in a tube to 150 µl 0.9 % sodium chloride solution and add 1 Cefotaxime+ Indicator (ESBL) and close the tube. **Vortex for 1–2 seconds to disintegrate the tablet.**

Incubate the test tube at 35-37 °C for **30 min** and if negative incubate **for 1 hour**, respectively.

The same process is repeated using the Cefotaxime + Tazobactam + Indicator Diatab.

**Blood cultures:**

Take 0.5 ml. of blood culture (positive for gram – negative bacilli) and add 100 ul of Saponin 5 % solution. Vortex. Maintain 5 min at room temperature. Centrifuge for 2 min. at 13.000 g. Discard the supernatant. Re-suspend the pellet in 500 ul distilled water and centrifuge at 13.000 g for 2 minutes. Discard the supernatant.

Re-suspend the bacterial pellet in a mixture of 150 ul 0.9 % NaCl solution + 50 µl TRIS – HCl lysis buffer and follow the procedure indicated. (Vortex for 1 min etc.) Follow the standard procedure.

Vasoo et al(3) when using the kit directly from blood cultures, found a **sensitivity** of 96.2 % and a **specificity** of 100 %.

**Urine samples:**

Take 10 ml urine (positive for gram – negative bacilli) and centrifuge. Suspend the bacteria pellet in a mixture of 150 ul Of 0.9 % NaCl solution + 50 µl TRIS – HCl lysis buffer and follow the procedure indicated.

**INTERPRETATION OF RESULTS:**

A change of color from red to yellow indicates a **positive reaction**.

If the reaction is positive after 15 minutes or 30 min, the test is finished (it is not necessary to incubate further), because positive reactions may fade out.

In a few cases an orange yellowish color or light yellow is obtained after incubation This is a positive result if the Cefotaxime+Tazobactam+Indicator (negative control) remains red.

If Cefotaxime+Indicator (ESBL) tube turns yellow and the Cefotaxime+Tazobactam+Indicator remains red the test is **positive for ESBL**.  
 If Cefotaxime + Indicator (ESBL) turns yellow and Cefotaxime+Tazobactam+Indicator also turns yellow, the test is **negative for ESBL**, but probably positive for a different beta-lactamase.  
 If both tubes maintain the red color: the test is **negative for ESBL**.  
 If the Cefotaxime + indicator is red, but the Cefotaxime+tazobactam+Indicator turns yellow, the test is uninterpretable.

**If the results are difficult to interpret:** use the following modifications: 1) holding the tube in vertical orientation above eye level and inspecting the bottom of the tablet, for yellow color (positive) and 2) the comparison of test and negative control tubes by viewing side by side, tilted gently to horizontal and examined in bright light above a white background. If the result remains unclear the test is repeated with higher inoculum.

Negative Control	Test	Report
Red or red-orange	Red or red-orange	ESBL negative
Red or red orange	Light orange,dark yellow/yellow	ESBL positive
Orange,dark yellow,yellow	Any colour	Invalid

**QUALITY CONTROL:**

DIATABS	Positive	Negative
	<i>Enterobacter cloacae</i> NCTC 13464	<i>E. coli</i> ATCC 25922
Cefotaxime + Indicator	<i>Yellow</i>	<i>Red</i>
Cefotaxime + Tazo + indicator	<i>Red</i>	<i>Red</i>

**REFERENCES:**

- 1) Nordmann P et al: Rapid detection of Extended-spectrum-beta-lactamase-producing Enterobacteriaceae. J.Clin Microbiol. 50, 3016-3022, 2012.
- 2) Dortet et al: Rapid detection of ESBL-producing Enterobacteriaceae in blood cultures. Emerging Infect Dis, 21, No 3, march 2015.
- 3) Vasoo Shawn et al :Accuracy of multiplex PCR plus a chromogenic phenotypic test algorithm for the detection of ESBLand carbapenemase-producing gram-negatives,directly from blood cultures.Singapore National Center for Infectious Diseases,paper 2289,October 2018.