## ROTI ${ }^{\circledR}$ Antibiotic Discs Polymyxin B (PB)

## For qualitative antimicrobial susceptibility testing (AST) of bacterial cultures 1495

## Appearance:

ROT ${ }^{\circledR}$ Antibiotic Discs are provided in cartridges of 50 discs each.
Polymyxin B 300 Units/Disc
Disc diameter 6 mm
Imprint (center of both sides)
PB 300
For research use only. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro diagnostics.

## STORAGE CONDITIONS

Store at $-20^{\circ} \mathrm{C}$ under dry conditions and protected from light immediately upon receipt.

## APPLICATION

## 1. Preparation of Inoculum

Prepare plates with Muller Hinton Agar (Art. No. X926). The medium in the plates should be sterile and have a depth of about 4 mm . Pick $4-5$ similar colonies with a wire, needle or loop and incubate in 5 ml of CASO Broth (Art. No. X938) at $35-37^{\circ} \mathrm{C}$ for $2-8 \mathrm{~h}$ until light to moderate turbidity. Adjust the turbidity to yield $1 \times 10^{6}-5 \times 10^{6} \mathrm{cfu} / \mathrm{ml}$ (i.acc. with $0,5 \mathrm{McFarland}$ standard (Art. No. 1440 or 1307 ) or $0,08-0,13$ OD at 625 nm ) by further incubating or by diluting.
2. Test Procedure (based on Bauer-Kirby method)

Dip a sterile non-toxic cotton swab on a wooden applicator (e.g. ROTILABO ${ }^{\circledR}$-cotton buds Art. No. EH12.1) into the standardised inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to gently remove excessing fluid. Streak the entire agar surface of the plate with the swab three times evenly, e.g. by turning the plate at $60^{\circ}$ angle between each streaking or using a petri dish revolving table (e.g. Art. No. N962.1) for guiding the plate evenly. Allow the inoculum to dry for 5-15 minutes with closed lid. The later growth on the plate should be semi-confluent.

Apply the discs using aseptic technique with sterile tweezers or the Antibiotic Disc Dispenser (Art. No. 1505.1). The centers of the discs should be at least $24-30 \mathrm{~mm}$ apart. Invert the plates and place in an incubator set to $35 \pm 2{ }^{\circ} \mathrm{C}$ within 15 minutes after the discs are applied.
It is recommended to prepare and incubate a plate with a known reference strain with known zone size for this antibiotic in parallel as a control.

Examine each plate after 16-18 hours of incubation or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time. The diameter of each zone with complete inhibition is measured e.g. using a calibrated pocket slide rule (Art. No. HCN3.1) and assessed according to valid databases.

## MICROBIOLOGICAL TEST

Average diameter of zone of inhibition observed on Muller Hinton Agar after 18 hours incubation at $35-37^{\circ} \mathrm{C}$ for standard cultures (strains recommended by CLSI (Clinical Laboratory Standards Institute)).

| Microorganisms | zone of diameter [mm] |
| :--- | :--- |
| E. coli ATCC 25922 | $13-19$ |
| Pseudomonas aeruginosa ATCC 27853 | $14-18$ |

*MHA is recommended to carry out susceptibility tests of rapidly growing MOs. Other MOs may require different media, e.g. MHA with $\mathrm{Ca}^{++} / \mathrm{Mg}^{++}$, Haemophilus Test Agar, MHA with blood, GC Agar. Choose carefully acc. to standard regulations.

## References:

Bauer et al (1966): Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Path. 45(4): 493-6.
CLSI: Performance standards of Antimicrobial Disc Susceptibility Tests. M02, Vol. 32 No.3, Jan 2012.

| ROTI®Antibiotic Discs Polymyxin B (PB) | $1 \times 50$ discs | 1495.2 |
| :--- | :--- | :--- |
|  | $5 \times 50$ discs | 1495.1 |

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[^0]:    Carl Roth GmbH + Co. KG
    Schoemperlenstraße 3-5•76185 Karlsruhe •P.O. Box 100121•76231 Karlsruhe
    Phone: +49 (0) 721/5606-0 • Fax: +49 (0) 721/5606-149 • info@carlroth.com • www.carlroth.com

