

# CHROMagar™ **ESBL**



## MEDIUM PURPOSE

Chromogenic medium for overnight detection of gram-negative bacteria producing extended spectrum beta-lactamase.

ESBL (Extended Spectrum β-Lactamases) are enzymes that mediate resistance to penicillins, extended-spectrum third generation cephalosporins (C3G) and monobactams. ESBL-producing Enterobacteriaceae started to appear in the 80s, and have since emerged as some of the most significant hospital-acquired infections with *Escherichia coli* and *Klebsiella* spp. being the main actors, but other gram-negative species have also been observed. Therefore, the early detection of ESBL-producing bacteria carriers is important to minimise their impact and the spread of infections and customise therapeutic patient treatment.

## COMPOSITION

The product is composed of a powder base (CHROMagar Orientation) and 1 supplement (CHROMagar ESB supplement).

Product	=	Base (RT)	+	Supplement (ES)
Total g/L		33.0 g/L		0.57 g/L
Composition g/L		Agar 15.0 Peptone and yeast extract 17.0 Chromogenic mix 1.0		Selective mix 0.57
Aspect		Powder Form		Powder Form
STORAGE		15-30°C		2/8°C
FINAL MEDIA pH		7.0 +/- 0.2		

## PREPARATION (Calculation for 1L)

<b>Step 1</b> Preparation of the base CHROMagar Orientation	<ul style="list-style-type: none"> <li>Disperse slowly 33g of powder base in 1L of purified water.</li> <li>Stir until agar is well thickened.</li> <li>Heat and bring to boiling (100°C) while swirling or stirring regularly.</li> </ul> <p style="color: green;">Advice 1: For enhanced growth, add 0.5g/L of Tween 80 to the previous preparation mix.</p> <p style="color: green;">Advice 2: For the 100°C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).</p>									
<b>Step 2</b> Autoclave	<ul style="list-style-type: none"> <li>AUTOCLAVE at 121°C during 15 min.</li> <li>Cool in a water bath to 45/50°C, swirling or stirring gently.</li> </ul>									
<b>Step 3</b> Preparation of the CHROMagar ESB supplement	<ul style="list-style-type: none"> <li>Weight 570 mg of the required supplement powder.</li> <li>Add 10ml of purified sterile water to this powder to make a supplement solution.</li> </ul> <p style="color: red;">Warning 1: This step may require several minutes of stirring to obtain a good and homogenous suspension: <b>opaque yellowish appearance.</b></p> <p style="color: red;">Warning 2: Reconstituted supplement solution must be used the same day.</p> <p style="color: red;">Warning 3: Do not store and re-use a supplement solution.</p>	<table border="1"> <thead> <tr> <th>Final Media</th> <th>HELPING CALCULATION</th> </tr> </thead> <tbody> <tr> <td>1 L</td> <td>Rehydrate 570 mg into 10 ml of purified water</td> </tr> <tr> <td>5 L</td> <td>Rehydrate 2,85 g into 50 ml of purified water</td> </tr> <tr> <td>25L</td> <td>Rehydrate 14,25 g into 250 ml of purified water</td> </tr> </tbody> </table>	Final Media	HELPING CALCULATION	1 L	Rehydrate 570 mg into 10 ml of purified water	5 L	Rehydrate 2,85 g into 50 ml of purified water	25L	Rehydrate 14,25 g into 250 ml of purified water
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<b>Step 4</b> Integrate the supplement to the melted base	<ul style="list-style-type: none"> <li>Vortex this supplement to homogenize and add this supplement solution to melted CHROMagar™ Orientation cooled at 45/50°C.</li> <li>Stir to make CHROMagar™ ESB.</li> </ul>									
<b>Step 5</b> Pouring	<ul style="list-style-type: none"> <li>Pour into sterile Petri dishes.</li> <li>Let it solidify and dry.</li> </ul>									
<b>Storage</b>	<ul style="list-style-type: none"> <li>Store in the dark before use.</li> <li>Prepared media plates can be kept for one day at room temperature.</li> <li>Plates can be stored for up to 1 month under refrigeration (2/8°C) if properly prepared and protected from light and dehydration.</li> </ul>									

## INOCULATION

Related samples can be processed by direct streaking on the plate.

- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.
- Incubate in aerobic conditions at 37°C for 18-24 hours.

## Typical Samples

e.g. stools, urine, rectal swabs  
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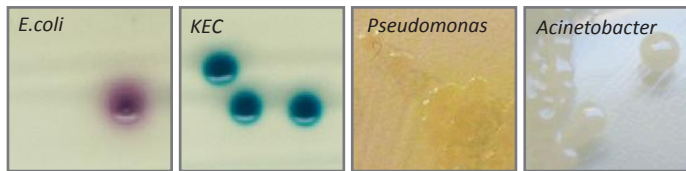
Direct streaking or spreading technique

# CHROMagar™ **ESBL**

## INTERPRETATION

Microorganism	Typical colony appearance
ESBL <i>E.coli</i>	→ dark pink to reddish
ESBL KEC ( <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Citrobacter</i> )	→ metallic blue
ESBL <i>Proteus</i>	→ brown halo
ESBL <i>Acinetobacter</i>	→ cream
ESBL <i>Pseudomonas</i>	→ translucent, (+/- natural pigmentation cream to green)
<i>Stenotrophomonas</i>	→ colourless
Gram(+) strains	→ inhibited
Non Resistant Other Gram(-) strains	→ inhibited
Yeasts	→ mostly inhibited

### Typical colony appearance



The pictures shown are not contractual.

## PERFORMANCE & LIMITATIONS

- Some *Pseudomonas* spp and *Acinetobacter* spp, widely-known to be frequently Multi Drug Resistant bacteria, could grow on the medium with typical colony aspects as Typical on CHROMagar™ Orientation.
- Final identification may require additional testing such as biochemical or immunological test: Latex agglutination confirmation test can be performed directly from the plates on suspected colonies.
- Most AmpC-producing bacteria are inhibited but some may show some growth.

## QUALITY CONTROL

Please perform Quality Control according to the use of the medium and the local QC regulations and norms. Good preparation of the medium can be tested, isolating the ATCC strains below:

Microorganism	Typical colony appearance
ESBL <i>E.coli</i> CIP 103982	→ reddish, small colonies
ESBL <i>K. pneumoniae</i> ATCC® 700603	→ metallic blue
<i>E.faecalis</i> ATCC® 29212	→ inhibited

Microorganism	Typical colony appearance
<i>P.aeruginosa</i> ATCC® 10145	→ inhibited
<i>E.coli</i> ATCC® 25922	→ inhibited
<i>C.albicans</i> ATCC® 60193	→ inhibited
<i>S.aureus</i> ATCC® 25923	→ inhibited

## WARNINGS

- Do not use plates if they show any evidence of contamination or any sign of deterioration.
- Do not use the product beyond its expiry date or if product shows any evidence of contamination or any sign of deterioration.
- For *in vitro* diagnostic use. This laboratory product should be used only by trained personnel in compliance with good laboratory practices.
- Any change or modification in the procedure may affect the results.
- Any change or modification of the required storage temperature may affect the performance of the product.
- Unappropriate storage may affect the shelf life of the product.
- Recap the bottles/vials tightly after each preparation and keep them in a low humidity environment, protected from moisture and light.
- For a good microbial detection: collection and transport of specimen should be well handled and adapted to the particular specimen according to good laboratory practices.

## DISPOSAL OF WASTE

After use, all plates and any other contaminated materials must be sterilized or disposed of by appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

## REFERENCES

Please refer to our website page «Publications» for scientific publications about this particular product.  
Web link: <http://www.chromagar.com/publication.php>

## IFU/LABEL INDEX

- Quantity of powder sufficient for X liters of media
- Expiry date
- Required storage temperature
- Store away from humidity

Pack Size	Ordering References	Base (RT)	Supplement (ES)
5000 ml <small>250 Tests of 20ml</small>	<b>ESRT2</b>	RT412 Weight: 165gr	ES372 Weight: 2,85gr
25 L <small>1250 Tests of 20ml</small>	<b>ESRT3-25</b>	RT413-25 Weight: 825gr	ES373-25 Weight: 14,25gr

### Need some Technical Documents?

- Available for download on [www.CHROMagar.com](http://www.CHROMagar.com)
- Certificate of Analysis (CoA) --> One per Lot
- Material Safety Data Sheet (MSDS)

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