CHROMagar™ ESBL

Instructions For Use
For Research Use Only (RUO).
Not for use in diagnostic procedures.

ENGLISH

Instructions For Use

REFERENCES

Pack Size		Ordering References	Base (RT)	Supplement (ES)
5000 mL	250 Tests of 20 mL	ESRT2	RT412 Weight: 165 g	ES372 Weight: 2,85 g
25 L	1250 Tests of 20 mL	ESRT3-25	RT413-25 Weight: 825 g	ES373-25 Weight: 14,25 g

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™ ESBL 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	

Need some Technical Documents?

Available for download on www.CHROMagar.com

- Certificate of Analysis (CoA) --> One per Lot
- Material Safety Data Sheet (MSDS)

PREPARATION **(Calculation for 1 L)**

Step 1
Preparation of the base CHROMagar™ Orientation

- Disperse slowly 33 g of powder base in 1 L of purified water.
- Stir until agar is well thickened.
- Heat and bring to boiling (100 °C) while swirling or stirring regularly.

Advice 1: For enhanced growth, add 0.5 g/L of Tween 80 to the previous preparation mix.
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).

Step 2
Autoclave

- AUTOCLAVE at 121 °C during 15 min.
- Cool in a water bath to 45/50 °C, swirling or stirring gently.

Step 3
Preparation of the CHROMagar™ ESBL supplement

- Weight 570 mg of the required supplement powder.
- Add 10 mL of purified sterile water to this powder to make a supplement solution.

Warning 1: This step may require several minutes of stirring to obtain a good and homogenous suspension: opaque yellowish appearance.
Warning 2: Reconstituted supplement solution must be used the same day.
Warning 3: Do not store and re-use a supplement solution.

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
25 L	Rehydrate 14,25 g into 250 mL of purified water

Step 4
Integrate the supplement to the melted base

- Vortex this supplement to homogenize and add this supplement solution to melted CHROMagar™ Orientation cooled at 45/50 °C.
- Stir to make CHROMagar™ ESBL.

Step 5
Pouring

- Pour into sterile Petri dishes.
- Let it solidify and dry.

Storage

- Store in the dark before use.
- Prepared media plates can be kept for one day at room temperature.
- Plates can be stored for up to 1 month under refrigeration (2/8 °C) if properly prepared and protected from light and dehydration.

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SPECIMEN COLLECTION AND HANDLING

CHROMagar™ ESBL can be used with the following specimens:
- In clinical field : stools, urine, perineal and rectal specimens.
- In veterinary field : livestock and poultry.

Sampling and transport equipment must be used in accordance with the recommendations of their suppliers for the conservation of *ESBL* strains.

MATERIAL REQUIRED BUT NOT PROVIDED

Standard microbiological laboratory material for culture media preparation, control, streaking, incubation and waste disposal.

INOCULATION

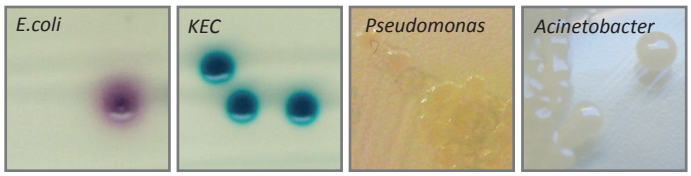
Related samples are inoculated by direct streaking on the plate.
- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.
- Incubate in aerobic conditions at 35-37 °C for 18-24 hours.

INTERPRETATION

Qualitative reading and interpretation of the petri dishes

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

Typical colony appearance



The pictures shown are not contractual.

PERFORMANCE

In the following study, 1552 rectal and oropharyngeal swabs were tested, being positive 394 after 18-24 h incubation (48-72h for reference method).

	CHROMagar™ ESBL	Reference Method (MacConkey Agar)
Sensitivity	98 % *	69 %
Specificity	97 % *	--

* Data obtained from the study «Detection of Extended-spectrum β- Lactamase producing Enterobacteriaceae» G. Klysova and al, ECCMID 2016

LIMITATIONS AND COMPLEMENTARY TESTS

- 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 following ATCC strains:

Microorganism	Typical colony appearance
ESBL *E. coli* CIP 103982	→ reddish, small colonies
ESBL *K. pneumoniae* ATCC® 700603	→ metallic blue
E. faecalis ATCC® 29212	→ inhibited
P. aeruginosa ATCC® 10145	→ inhibited
E. coli ATCC® 25922	→ inhibited
C. albicans ATCC® 60193	→ inhibited
S. aureus ATCC® 25923	→ inhibited

WARNINGS AND PRECAUTIONS

- For Research Use Only (RUO). Not for use in diagnostic procedures.
- This laboratory product should be used only by trained personnel (healthcare professional, etc). Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with procedures and good laboratory practices.
- Use of the medium may be difficult for people who have problems recognising colours.
- Culture media should not be used as manufacturing material or components.
- Do not ingest or inhale the product.
- Do not use the product after the expiry date.
- Do not use the product if it shows any evidence of contamination or any sign of deterioration (compacted powder, color change, ...).
- Do not use the product if the packaging is damaged.
- Any change or modification in the production 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.
- Do not use the culture medium poured into a petri dish after a first use.
- After opening the bottles and with an appropriate conservation, open bottles can be used under the same conditions until each product's expiry date.
- Reading and interpretation should be performed using isolated colonies.
- Some precipitates may be observed in the agar but these do not affect the performance of the product.

• Interpretation of the test results should be made taking into consideration colonial and microscopic morphology and if necessary, the results of any other tests performed.
• Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with all local and national regulations.
• For hazard and precaution recommendations related to some chemical components in this medium, please refer to the pictogram(s) mentioned on the labels. The Material Safety Data Sheet (MSDS) is available on www.chromagar.com
• Any incident or complaint related to the environment must be declared to the manufacturer at the following email address: chromagar@chromagar.com
• Any serious incident occurring in connection with the environment must be declared to the competent authorities and to the manufacturer at the following email address: chromagar@chromagar.com

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.

LITERATURE 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

Catalogue reference


Consult instructions for use


Quantity of powder sufficient for X liters of media


Expiry date


Required storage temperature


Store away from humidity


Protect from light


Manufacturer

NT-EXT-50 USA V7.0 / 27-May-20

CHROMagar™
The Chromogenic Media Pioneer

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