**INSTRUCTIONS FOR USE**

ChromArt

# ESBL SUPPLEMENT

**Freeze-dried selective supplement**

## 1 - INTENDED USE

*In vitro* diagnostics. Mixture of antimicrobials to be added to CRE-ESBL Base for the presumptive determination of ESBL-producing *Enterobacteriaceae*, in clinical specimens.

## 2 - COMPOSITION - VIAL CONTENTS

| | |
|---|---|
| Antimicrobials mix | 0.21 g |

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

ESBL Supplement added to CRE-ESBL Base, can be used for the preparation of ESBL medium for the isolation and differentiation of ESBL-producing *Enterobacteriaceae.*

The use of chromogenic media is the preferred option for the detection of ESBL-producers in faecal screening.[1,2]

Bacterial differentiation is obtained with a mixture of chromogenic compounds designed to detect specific enzymatic activities (β-galactosidase, β-glucosidase, tryptophanase), of *E.coli,* of bacteria of the KESC group (*Klebsiella, Enterobacter, Serratia, Citrobacter*) and of the *Proteus-Morganella-Providencia* group. The grey and opaque background of the medium allows a better observation and colour reading of the colonies.

The selectivity of the medium is due to the presence of an inhibitory mixture of antibiotics against Gram-positive bacteria, fungi and Gram-negative bacteria susceptible to 3rd or 4th generation cephalosporins.

## 4- DIRECTIONS FOR MEDIUM PREPARATION

Dissolve the contents of one vial of ESBL Supplement with 5 mL of sterile purified water. Add to 500 mL of CRE-ESBL Base (REF 408025) autoclaved and cooled to 47-50°C under aseptic conditions. Mix well and distribute into sterile Petri dishes.

## 5 - PHYSICAL CHARACTERISTICS

| | |
|---|---|
| Appearance of the lyophilized | high, homogeneous, reddish pastille |
| Appearance of the solution | opalescent reddish |

## 6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| ChromArt ESBL Supplement | Freeze-dried supplement | 4240080 | 10 vials, each for 500 mL of medium base |

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

CRE-ESBL Agar Base (REF 408025), autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

## 8 - SPECIMENS

ESBL medium is intended for screening clinical specimens such as stools, rectal or peri-rectal swab and for processing other clinical specimens such as urine, wounds and respiratory secretions.[1]

Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; collect specimens before antimicrobial therapy where possible.

## 9- TEST PROCEDURE

Allow plates to come to room temperature. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in air at 35-37°C for 18-24 hours.

## 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

ESBL producing *Enterobacteriaceae* show the following characteristic colonies:

Pink / red-magenta colonies: *E.coli*

Blue / green-blue / blue-violet / grey-violet colonies: *Klebsiella, Enterobacter, Serratia, Citrobacter*

Brown colonies with brown halo: *Proteus-Morganella-Providencia*

ESBL producers shall be subjected to confirmatory tests. Consult the listed references.[1-3]

## 11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

| CONTROL STRAINS | | INCUBATION T°/ T / ATM | EXPECTED RESULTS |
|---|---|---|---|
| *K. pneumoniae* SHV-18 | ATCC 700603 | 35-37°C / 18-24H / A | growth with blue colonies |
| *E. coli* | ATCC 25922 | 35-37°C / 18-24H / A | inhibited |
| *C. albicans* | ATCC 10231 | 35-37°C / 18-24H / A | inhibited |

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A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

## 12- PERFORMANCES CHARACTERISTICS

The performances of ESBL medium prepared with CRE-ESBL Base and ESBL Supplement were evaluated in a clinical study by a Clinical Microbiology Laboratory in northern Italy[4] on 2500 urine cultures and 38 samples from other body sites.
The data demonstrate the capacity of ESBL Medium to detect ESBL-producing *Enterobacteriaceae* with high sensitivity (98.82%) and specificity (98.29%).

Prior to release for sale representative samples of dehydrated ChromArt CRE-ESBL Base REF 408025, supplemented with ChromArt ESBL Supplement REF 4240080 are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.Productivity is tested by semi-quantitative ecometric technique with the following target strains: *K.pneumoniae* ATCC 700603, ESBL-producing clinical isolates of *E.coli, E.cloacae, C.freundii*. and *C. koserii*. After incubation at 35-37°C for 18-24 hours all target strains show a good growth with typical chromatic characteristics.
Selectivity is evaluated by semi-quantitative ecometric technique by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms *P.aeruginosa* ATCC 27853, *C.albicans* ATCC 10231, *S.aureus* (MR) ATCC 43300, *E.coli* ATCC 25922, *A.calcoaceticus* ATCC 19606, *E.fecium* (VRE) ATCC 700221, a clinical isolate of *E.cloacae* hyperproducer of AmpC and por+, a clinical isolate of *E.coli* hyperproducer of AmpC.
After incubation at 35-37°C for 18-24 hours, the growth of *P.aeruginosa, C.albicans* , *S.aureus, E coli* ATCC 25922 *and E faecium* is totally inhibited while the growth of hyperproducer of AmpC non-target strains *E.coli* and *E.cloacae* is partially inhibited.

## 12-LIMITATIONS OF THE METHOD

- ESBL Chromogenic agar media are likely to be less specific, particularly in areas where ESBL producers are common.[1]
- Some *Enterobacteriaceae* strains hyperproducing cephalosporinases, some multi drug resistant *Pseudomonas* spp. and *Acinetobacter* spp. may grow on the ESBL medium.
- Growth on the medium depends on the metabolic requirements of each microorganism and on the resistance to the antimicrobials present; some target strains may not be able to grow on ESBL medium or may show a delayed growth (e.g., *Proteus* spp.).
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. On the isolates, perform antimicrobial susceptibility testing.
- ESBL Supplement and the complete medium are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

## 14 - PRECAUTIONS AND WARNINGS

- ESBL Supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- ESBL Supplement is classified as dangerous according to current European legislation; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the preparation process of plated media.
- ESBL Supplement is sterilized by membrane filtration.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use ESBL Supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

## 15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).
The user is responsible for the manufacturing and quality control processes of plated media and the validation of their shelf life, according to the applied storage conditions (temperature and packaging).

## 16 - REFERENCES

1. Public Health England. UK Standards for Microbiology Investigations (SMI) B 59: Detection of *Enterobacteriaceae* producing extended spectrum β lactamases.2016
2. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev. 2017; 30:449-479.
3. Simner PJ, Humphries R. Special phenotypic methods for detecting antibacterial resistance. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. Comi C, Bracco S, Colombo L, Bartesaghi P, Barletta R, Silva M, Luzzaro F. Valutazione del terreno ChromArt ESBL (Biolife) per la rilevazione degli Enterobatteri produttori di ESBL in campioni clinici. XLIII Congresso AMCLI, Sezione Poster, 2014.

**4240080 ESBL SUPPLEMENT**
SDS
Regulation (EU) 2020/878

**Contains:**
CEFSULODINE
CLOXACILLIN SODIUM
CEFPODOXIME SODIUM

**Classification**

| | | |
|---|---|---|
| Respiratory sensitization, category 1 | H334 | May cause allergy or asthma symptoms or breathing difficulties if inhaled. |
| Skin sensitization, category 1 | H317 | May cause an allergic skin reaction. |

**Labelling**
Hazard pictograms:

Signal words: Danger
Hazard statements:
**H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled.
**H317** May cause an allergic skin reaction.
Precautionary statements:
**P261** Avoid breathing dust / fume / gas / mist / vapours / spray.
**P280** Wear protective gloves.
**P342+P311** If experiencing respiratory symptoms: Call a POISON CENTER / doctor / . . .
**P304+P340** IF INHALED: Remove person to fresh air and keep comfortable for breathing.
**P333+P313** If skin irritation or rash occurs: Get medical advice / attention.
**P362+P364** Take off contaminated clothing and wash it before reuse.

**TABLE OF APPLICABLE SYMBOLS**

| | | | | | |
|---|---|---|---|---|---|
| REF or REF Catalogue number | LOT Batch code | IVD *In vitro* Diagnostic Medical Device | Manufacturer | This side up | |
| Temperature limitation | Content sufficient for <n> tests | Consult Instructions for Use | Use by | Keep away from direct light | Fragile |

**REVISION HISTORY**

| Version | Description of changes | Date |
|---|---|---|
| Revision 2 | Updated layout and contents | 2021/12 |
| Revision 3 | Removal of obsolete classification | 2023/04 |
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Note: minor typographical, grammatical, and formatting changes are not included in the revision history.