

Test for *Escherichia coli* and Coliforms in water
Specific Enzyme Substrate Culture Medium Method
(Xgal method / Xgal-MUG medium with Sodium Pyruvate)

EC Blue 100P “Nissui”

EC Blue 100 “Nissui”

EC Blue 10 “Nissui”

Background

Both The Ministry of Health, Labor and Welfare (MHLW) in 2003 and Japan Water Works Association (JWWA) in 2001 officially approved EC Blue as one of the official methods for detection of *Escherichia coli* and Coliforms in water. EC Blue was developed based on the Specific Enzyme Substrate Culture Medium Method that is used specific enzyme substrate for detection of *E. coli* and Coliforms in water.

Colorimetric enzyme substrate, Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) in the medium is decomposed to bring out Blue/Blue-Green color by β-galactosidase specifically generated by coliforms. Also MUG (4-Methyl-Umbelliferyl-β-D-Glucuronide) in the medium is decomposed by β-Glucuronidase that *E. coli* generates specifically, to isolate a fluorescence substrate of 4-Methyl-Umbelliferone.

EC Blue series are packed in special snap package (EC Blue 100P), a disposable bottle (EC Blue 100) or a disposable tube (EC Blue 10). These packages are sterilized by electron irradiation; therefore preparation of medium is not necessary. Test procedure of EC Blue 100P is very simple that only add it into test sample. Also test procedure of EC Blue 100 and 10 is simple that add sample water into these bottles (tubes).

Features

- 1) One medium can detect both *E. coli* and Coliforms sensitively.
- 2) It can confirm *E. coli* and Coliforms without any additional test.
- 3) Preparation and sterilization of medium are not necessary. Operation and judgment is very simple.
- 4) It can be judged after 24 hours incubation.
- 5) Medium is granule and easy to dissolve in water.
- 6) It is easy to confirm *E. coli* because special bottle without generating fluorescence is used (EC Blue 100 and 10).
- 7) It is selectable from 3 different types of package according to test purpose.

Kit Composition

EC Blue 100P “Nissui”

Sterilized granule medium in special snap pack ······ For 100 mL (1.74 g) × 100 pcs

Granule medium is packed in special snap package and sterilized by electron irradiation; therefore preparation of medium is not necessary. A sterilized container for testing is necessary separately.

EC Blue 100 “Nissui”

Sterilized granule medium in the bottle ······ For 100 mL (1.74 g) × 80 bottles

Granule medium is packed in special disposable bottle and sterilized by electron irradiation; therefore there is no need of medium preparation and a sterilized container.

EC Blue 10 “Nissui”

Sterilized granule medium in the tube ······ For 10 mL (0.17 g) × 150 tubes

Granule medium is packed in special disposable tube and sterilized by electron irradiation; therefore there is no need of medium preparation and a sterilized container.

Composition of the medium

For 1 liter = 17.4 g

Peptone ······	5.0 g
Sodium chloride ······	5.0 g
Monopotassium phosphate ······	1.0 g
Dipotassium phosphate ······	4.0 g
Sodium lauryl sulfate ······	0.1 g
Sodium pyruvate ······	1.0 g
Potassium nitrate ······	1.0 g
Isopropyl-β-D-Thiogalactopyranoside (IPTG) ······	0.1 g
5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal) ······	0.1 g
4-Methyl-Umbelliferyl-β-D-glucuronide (MUG) ······	0.1 g
	ph 7.1 ±

Purpose of Test

Detection of *E. coli* and Coliforms in water (EC Blue 100 and 100P), Quantitative test (MPN) of *E. coli* and Coliforms in water (EC Blue 10)

Detection Principle

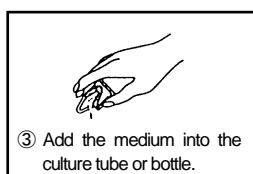
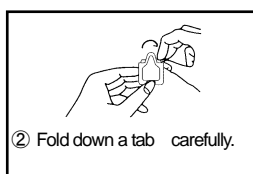
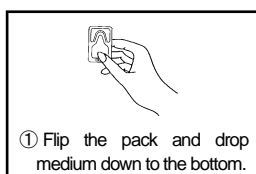
Coliforms : Colorimetric enzyme substrate, Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) in the medium is decomposed to bring out Blue /Blue-Green color by β-galactosidase specifically generated by coliforms. Result is judged under natural light.

E. coli : MUG (4-Methyl-Umbelliferyl-β-D-Glucuronide) in the medium is decomposed by β-glucuronidase that *E. coli* generates specifically, to isolate a fluorescence substrate of 4-Methyl-Umbelliferone that fluorescent under UV lamp (365nm).

Operating Procedure

1. EC Blue 100P “Nissui” ······ For 100 mL qualitative test

- 1) Detach one piece of Snap pack from 5 coupling snap packs.
- 2) Prepare sterilized culture tube (bottle). Dispense 100 mL of clean caught specimen from collection bottle into sterilized culture tube (bottle).
- 3) Refer to the following figures and add one pack of EC Blue 100P into the culture tube (bottle). Tighten the cap and well swirl for dissolving the medium in water sample.
- 4) Judge color change (Blue - Blue Green) of the medium after 24 hour incubation at 35 - 37°C. When



confirm Blue-Blue Green color change, check fluorescence additionally (for detection of *E. coli*).

2. EC Blue 100 “Nissui” ······ For 100 mL qualitative test

- 1) Prepare EC Blue 100 (bottle) and dispense 100mL of test sample into the bottle under sterilized condition. The bottle is graduated in 100 mL mark on circumference.
- 2) Tighten the cap and well swirl for dissolving the medium in water sample.
- 3) Judge color change (Blue - Blue Green) of the medium after 24 hours incubation at 35 - 37°C. When confirm Blue-Blue Green color change, check fluorescence additionally (for detection of *E. coli*).

3. EC Blue 10 “Nissui” ······ For 10 mL quantitative (MPN) test

- 1) Prepare 10-fold dilution series aseptically (refer to MPN method in the official documents issued).
- 2) Dispense diluted 10 mL sample in each tube. The tube is graduated in 10 mL mark on circumference.
- 3) Tighten the cap and well swirl for dissolving the medium in water sample.

- 4) Judge color change (Blue - Blue Green) of the medium after 24 hours incubation at 35 - 37°C. When confirm Blue-Blue Green color change, check fluorescence additionally (for detection of *E. coli*).

Precaution for use

- 1) Preparation of Apparatus and Materials
 - Incubator (35 - 37°C)
 - UV lamp (365 nm) (Mini UV lamp :Code 05614)
 - Sterilized culture tube or bottle (for EC Blue 100P)
 - Tube rack (for EC Blue 10)
- 2) Be careful to avoid any contamination and collect specimen aseptically.
- 3) Do not use the product when medium color is changed to brown or medium becomes solidified.
- 4) Be careful to avoid any scattering of medium when add or dispense specimen into the tube or the bottle.
- 5) When dispense the specimen into EC Blue 100 or EC Blue 10, unscrewed the cap of bottle or tube beforehand.
- 6) Keep the products away from direct sunlight, because colorimetric enzyme substrate may be decomposed by sunlight and it may give false positive result.
- 7) *E. coli* O-157 cannot be detected as *E. coli* by EC Blue series, because *E. coli* O-157 does not generate β-Glucuronidase.

Interpretation

- 1) For Coliforms: If color of cultured medium (Blue - Blue Green) is darker or same as EC Blue color comparator under natural light, determine the test result as “Coliforms positive”.
- 2) For *E. coli*: When confirm Blue-Blue Green color change; check fluorescence additionally under 365nm UV lamp. If fluorescence of the sample is stronger or same as EC Blue comparator under 365nm UV lamp, determine the test result as “*E. coli* positive”.

Precaution for interpretation

- 1) In case a sterilized culture tube (bottle) is used for the test (EC Blue 100P users), EC Blue color comparator recommended to transfer into the same culture tube (bottle) for judgment of the test result.
- 2) In case well water and / or river water that containing many heterotrophic bacteria is tested, cultured medium after incubation might be turbid. Also hard water might be turbid, but it may not be interfere judgment. In case there are some difficulties to read color reaction, observe the bottom of culture tube (bottle) or top surface of the medium for judgment.
- 3) Color change of coliform are typically Blue - Blue Green, but in some cases, medium will be colored to Green or Yellow Green by some Coliforms.

Performance

Inoculate 6 tested strains into 10 tubes of EC Blue 10, LB and BGLB media with 1cfu/tube. Test result (color change) after 24 hours incubation is listed on the right. (For Coliforms detection)

Also fluorescence of those media is indicated in brackets (For *E. coli* detection, EC Blue only).

Name of tested strains	EC Blue	LB	BGLB
<i>E. coli</i> ATCC 11775	10 (10)	7	6
<i>C. freundii</i> ATCC 8090	10 (0)	9	1
<i>E. cloacae</i> ATCC 13047	4 (0)	0	0
<i>K. pneumoniae</i> ATCC 13883	7 (0)	3	0
<i>A. hydrophila</i> JCM 1027	0 (0)	0	0
<i>R. aquatilis</i> JCM 1683	7 (0)	1	0

Warning and Direction for use

1. General Precautions

- 1) Read and follow precisely warning and direction for use described on this package insert and /or label.
- 2) Do not use this product that contains any foreign materials or particle caused by damaged container.
- 3) Do not use the product after its expiry date. Quality of the product is not warranted after its expiry date.
- 4) After opening the case, color of granule medium may be changed by effectiveness of lights. Performance of EC Blue is not be influenced by this medium color change.

2. Precautions for danger

- 1) When if medium or reagent touched eyes or mouths, immediately wash with plenty of water; and consult a physician.
- 2) Manipulations with microorganisms involve always certain risks of laboratory-acquired infections. Manipulations should be practiced under the supervision of key specialist with biohazard protection measures.
- 3) Any laboratory equipment and test reagent, medium that touched with specimen should be regarded as infectious in the laboratory.

3. Precautions for disposal of waste

Any media, reagents and materials used must be sterilized by autoclaving or boiling in water. Also dispose these waste as industrial or medical waste according to the local laws and regulations related to dispose.

4. Limitation of Warranties

If EC Blue has proven to defective, Nissui Pharmaceutical or Nissui’s authorized distributor will replace or refund at the purchase price of the product.

Storage and Shelf life

Storage

Keep at Room Temperature (1 -30°C), in dark

Shelf life

* EC Blue 100P “Nissui” Two (2) years after manufacturing

* EC Blue 100 “Nissui” Two (2) years after manufacturing

EC Blue 10 “Nissui” One (1) year after manufacturing

Package

EC Blue 100P “Nissui” For 100 mL × 100 packs ······	Code 05591
EC Blue 100 “Nissui” For 100 mL × 80 bottles ······	Code 05593
EC Blue 10 “Nissui” For 10 mL × 150 tubes ······	Code 05613

Related products

Mini UV lamp ······	Code 05614
EC Blue 100 color comparator “Nissui” For 100 mL × 1 bottle ······	Code 05617
EC Blue 10 color comparator “Nissui” For 10 mL × 1 tube ······	Code 06516

Storage: Keep at 4 -10°C, light shielding
Shelf life: One (1) year after manufacturing

Further information

Customer Support Section,

Nissui Pharmaceutical Co., Ltd.

3-23-9, Ueno, Taito-ku, Tokyo 110-8736 Japan

TEL: +81-3-5846-5707 / FAX: +81-3-5846-5629

E-mail: customer@nissui-pharm.co.jp

Manufactured by

NISSUI PHARMACEUTICAL CO., LTD.

3-23-9, Ueno, Taito-ku, Tokyo 110-8736 Japan

Revised: November 2007