

Instructions for use



EC Blue 10, 100, 100P

Made by NISSUI

For qualitative and quantitative detection of Coliforms and *E. coli* in particular in water.

A. Introduction

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.

Colourimetric enzyme substrate, X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) in the medium is decomposed to bring out Blue/Blue -Green colour 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 which may be detected using UV light of 365 nm.

The EC Blue 10 system is particularly recommended for quantitative measurement by the MPN method using the EC Blue Quant/MPN device (Art. No. 0199.1).

B. Composition of the granulated powder

17.4 g/l, pH 7.1

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

C. Application

C.1. EC Blue 100P - for standard 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 colour change (blue – blue/green) of the medium after 24 hour incubation at 35-37 °C. When blue-blue/green colour change is confirmed, additionally check fluorescence (for detection of *E. coli*).

C.2. EC Blue 100 - for ready-to-use 100 ml qualitative test

- 1) Prepare EC Blue 100 (bottle) and dispense 100ml of test sample into the bottle under sterile 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 colour change (blue – blue/green) of the medium after 24 hour incubation at 35-37 °C. When blue-blue/green colour change is confirmed, additionally check fluorescence (for detection of *E. coli*).

C.3. EC Blue 10 - for ready-to-use 10 ml qualitative test

- 1) *Optional*: Prepare 10-fold dilution series aseptically (if required, 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 colour change (blue – blue/green) of the medium after 24 hour incubation at 35-37 °C. When blue-blue/green colour change is confirmed, additionally check fluorescence (for detection of *E. coli*).

For quantitative testing, the EC Blue Quant/MPN device (Art. No. 0199.1) should be used. Please refer to the instructions-for-use there.

D. Interpretation of results

- 1) For Coliforms: If colour of cultured medium (blue to blue/green) is darker or same as EC Blue colour comparator under natural light, determine the test result as “Coliforms positive”.
- 2) For *E. coli*: In case of blue to blue/green colour change, additionally check fluorescence under 365 nm UV lamp. If fluorescence of the sample is stronger or same as EC Blue comparator under 365 nm UV lamp, determine the test result as “*E. coli* positive”.

Please note:

- A) In case a sterilized culture tube (bottle) is used for the test (EC Blue 100P users), EC Blue colour comparator has to be transferred into a similar culture tube (bottle) for judgment of the test result.
- B) In case well water or river water containing many heterotrophic bacteria is to be tested, the medium might be turbid after incubation. Also hard water might be turbid, but without interference with interpretation of the results. In case colour reaction is not easy to read, observe the bottom of culture tube (bottle) or top surface of the medium for judgment.
- C) Colour change of coliforms is typically blue to blue/green, but some Coliforms are known to result in a more green or yellow/green colour of the medium.

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The company is a limited partnership with headquarters in Karlsruhe, reg. court Mannheim HRA 100055. Roth Chemie GmbH, with headquarters in Karlsruhe, reg. court Mannheim HRB 100428, is the personally liable partner. Managing Director: André Houdelet. Sales tax identification number: DE 143621073.

E. Precautions for use

- 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, colour of the granulated medium may change due to the influence of light. In most cases, performance of EC Blue is not influenced by this medium colour change. However, do not use the product when medium colour is changed to strong brown or if the medium becomes solidified.
- 5) Be careful to avoid any contamination and collect specimen aseptically.
- 6) Be careful to avoid any scattering of medium when add or dispense specimen into the tube or the bottle.
- 7) When dispense the specimen into EC Blue 100 or EC Blue 10, unscrewed the cap of bottle or tube beforehand.
- 8) Keep the products away from direct sunlight, because colourimetric enzyme substrate may be decomposed by sunlight and it may give false positive result.
- 9) *E. coli* O:157 cannot be detected as *E. coli* by EC Blue series, because *E. coli* O:157 does not generate β -Glucuronidase.

F. Quality control

Six test strains are inoculated into 10 tubes of EC Blue 10, LB and BGLB media with 1 cfu per tube. Test result (colour change, for detection of Coliforms) after 24 hours incubation is listed.

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

Test organism	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

G. Safety precautions and disposal of waste

- 1) For safety recommendations please refer to the Safety Data Sheet provided on our webpages.
- 2) Manipulations with microorganisms always involve certain risks of laboratory-acquired infections. Application of these products should be practiced under the supervision of key specialist with biohazard protection measures.
- 3) Any laboratory equipment and test reagent or medium that has been touched by the waters to be tested should be regarded as infectious in the laboratory.
- 4) Following testing, any media, materials and apparatuses have to be sterilized by autoclaving equivalent procedures for full decontamination of microorganisms. Then dispose as medical or industrial waste according to the regulations on Waste Disposal and Cleaning given by your country or institution.

H. Shipment, storage and shelf life

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). All packages are sterilized by electron irradiation; therefore preparation of medium is not necessary.

Keep at room temperature protected from light.

Use best before one (1) year after manufacturing.

EC Blue 100 P	100 pieces	0202.1
EC Blue 100	80 pieces	0209.1
EC Blue 10	150 pieces	7754.1

MPN calibration scale

Note: The MPN calibration scale is based on the program developed in 1985 by Hurley & Roscoe. This program makes provision for the reliability coefficient to evaluate the reliability of data. In case that the combination of positive number indicates <9 of the reliability coefficient, MPN is valid. On the other hand, in case of >9, MPN may not be valid by some sort of error cause. MPN indicated in italic letter should be handled as a reference value because of >9.

Positive Number			MPN in 100 ml	95% confidence limit		Positive Number			MPN in 100 ml	95% confidence limit		Positive Number			MPN in 100 ml	95% confidence limit	
10 ml	1 ml	0.1 ml		lower limit	upper limit	10 ml	1 ml	0.1 ml		lower limit	upper limit	10 ml	1 ml	0.1 ml		lower limit	upper limit
0	0	0	0	0	0	1	0	0	2,0	0,28	14	2	0	0	4,5	1,1	18
0	0	1	1,8	0,23	14	1	0	1	4,0	0,94	17	2	0	1	6,8	2,1	22
0	0	2	3,6	0,80	16	1	0	2	6,0	1,7	21	2	0	2	9,1	3,1	27
0	0	3	5,4	1,5	20	1	0	3	8,1	2,6	25	2	0	3	12	4,1	32
0	0	4	7,2	2,3	23	1	0	4	10	3,5	29	2	0	4	14	5,2	37
0	0	5	9,0	3,1	27	1	0	5	12	4,4	34	2	0	5	16	6,2	43
0	1	0	1,8	0,24	14	1	1	0	4,0	0,95	17	2	1	0	6,8	2,1	22
0	1	1	3,6	0,81	16	1	1	1	6,1	1,8	21	2	1	1	9,2	3,1	27
0	1	2	5,5	1,5	20	1	1	2	8,1	2,7	25	2	1	2	12	4,2	32
0	1	3	7,3	2,3	23	1	1	3	10	3,6	29	2	1	3	14	5,2	38
0	1	4	9,1	3,1	27	1	1	4	12	4,5	34	2	1	4	17	6,3	44
0	1	5	11	3,9	31	1	1	5	14	5,4	39	2	1	5	19	7,3	50
0	2	0	3,7	0,82	16	1	2	0	6,1	1,8	21	2	2	0	9,3	3,2	27
0	2	1	5,5	1,5	20	1	2	1	8,2	2,7	25	2	2	1	12	4,2	33
0	2	2	7,4	2,3	23	1	2	2	10	3,6	29	2	2	2	14	5,3	38
0	2	3	9,2	3,1	27	1	2	3	12	4,5	34	2	2	3	17	6,4	44
0	2	4	11	3,9	31	1	2	4	15	5,4	39	2	2	4	19	7,4	51
0	2	5	13	4,7	35	1	2	5	17	6,3	44	2	2	5	22	8,4	58
0	3	0	5,6	1,6	20	1	3	0	8,3	2,7	25	2	3	0	12	4,3	33
0	3	1	7,4	2,3	23	1	3	1	10	3,6	30	2	3	1	14	5,4	39
0	3	2	9,3	3,2	27	1	3	2	13	4,6	34	2	3	2	17	6,5	45
0	3	3	11	4,0	31	1	3	3	15	5,5	39	2	3	3	20	7,5	52
0	3	4	13	4,8	35	1	3	4	17	6,4	44	2	3	4	22	8,5	59
0	3	5	15	5,6	40	1	3	5	19	7,3	50	2	3	5	25	9,4	67
0	4	0	7,5	2,4	20	1	4	0	11	3,7	30	2	4	0	15	5,5	39
0	4	1	9,4	3,2	27	1	4	1	13	4,6	35	2	4	1	17	6,5	45
0	4	2	11	4,0	31	1	4	2	15	5,6	40	2	4	2	20	7,6	52
0	4	3	13	4,8	36	1	4	3	17	6,5	45	2	4	3	23	8,6	60
0	4	4	15	5,6	40	1	4	4	19	7,3	51	2	4	4	25	9,5	68
0	4	5	17	6,4	45	1	4	5	22	8,2	56	2	4	5	28	10	76
0	5	0	9,4	3,2	28	1	5	0	13	4,7	35	2	5	0	17	6,6	46
0	5	1	11	4,1	32	1	5	1	15	5,6	40	2	5	1	20	7,7	53
0	5	2	13	4,9	36	1	5	2	17	6,5	45	2	5	2	23	8,7	60
0	5	3	15	5,7	40	1	5	3	19	7,4	51	2	5	3	26	9,6	69
0	5	4	17	6,5	45	1	5	4	22	8,3	57	2	5	4	29	11	78
0	5	5	19	7,2	50	1	5	5	24	9,1	64	2	5	5	32	11	87

Positive Number			MPN in 100 ml	95% confidence limit		Positive Number			MPN in 100 ml	95% confidence limit		Positive Number			MPN in 100 ml	95% confidence limit	
10 ml	1 ml	0.1 ml		lower limit	upper limit	10 ml	1 ml	0.1 ml		lower limit	upper limit	10 ml	1 ml	0.1 ml		lower limit	upper limit
3	0	0	7,8	2,5	24	4	0	0	13	4,7	35	5	0	0	23	8,8	61
3	0	1	11	3,7	30	4	0	1	17	6,3	44	5	0	1	31	11	87
3	0	2	13	5,0	36	4	0	2	21	7,9	54	5	0	2	43	14	130
3	0	3	16	6,2	44	4	0	3	25	9,5	67	5	0	3	58	18	180
3	0	4	20	7,5	51	4	0	4	30	11	83	5	0	4	76	25	230
3	0	5	23	8,7	60	4	0	5	36	12	100	5	0	5	95	33	280
3	1	0	11	3,8	30	4	1	0	17	6,4	45	5	1	0	33	12	92
3	1	1	14	5,1	37	4	1	1	21	8,1	56	5	1	1	46	15	140
3	1	2	17	6,3	44	4	1	2	26	9,7	69	5	1	2	63	20	200
3	1	3	20	7,6	52	4	1	3	31	11	85	5	1	3	84	28	250
3	1	4	23	8,8	61	4	1	4	36	13	100	5	1	4	110	37	300
3	1	5	27	10	72	4	1	5	42	14	130	5	1	5	130	47	350
3	2	0	14	5,1	37	4	2	0	22	8,2	57	5	2	0	49	16	150
3	2	1	17	6,4	45	4	2	1	26	9,9	71	5	2	1	70	22	220
3	2	2	20	7,7	53	4	2	2	32	11	88	5	2	2	94	32	280
3	2	3	24	8,9	62	4	2	3	38	13	110	5	2	3	120	43	330
3	2	4	27	10	73	4	2	4	44	15	130	5	2	4	150	55	400
3	2	5	31	11	85	4	2	5	50	16	160	5	2	5	180	66	470
3	3	0	17	6,5	45	4	3	0	27	10	73	5	3	0	79	26	240
3	3	1	21	7,8	54	4	3	1	33	12	91	5	3	1	110	38	310
3	3	2	24	9,1	64	4	3	2	39	13	110	5	3	2	140	52	380
3	3	3	28	10	74	4	3	3	45	15	140	5	3	3	170	66	470
3	3	4	31	11	86	4	3	4	52	17	160	5	3	4	210	80	560
3	3	5	35	12	100	4	3	5	59	19	190	5	3	5	250	93	680
3	4	0	21	7,9	55	4	4	0	34	12	94	5	4	0	130	47	360
3	4	1	24	9,2	65	4	4	1	40	14	120	5	4	1	170	65	460
3	4	2	28	10	76	4	4	2	47	15	140	5	4	2	220	83	590
3	4	3	32	12	88	4	4	3	54	17	170	5	4	3	280	100	760
3	4	4	36	13	100	4	4	4	61	20	190	5	4	4	350	120	1000
3	4	5	40	14	120	4	4	5	69	22	220	5	4	5	430	140	1300
3	5	0	25	9,3	66	4	5	0	41	14	120	5	5	0	240	89	640
3	5	1	29	11	77	4	5	1	48	16	150	5	5	1	350	12	1000
3	5	2	32	12	90	4	5	2	56	18	180	5	5	2	540	160	1800
3	5	3	37	13	100	4	5	3	64	20	200	5	5	3	920	290	2900
3	5	4	41	14	120	4	5	4	72	23	220	5	5	4	1600	540	4800
3	5	5	45	15	140	4	5	5	81	27	240	5	5	5	≥2400		