



# Instructions for use

## EC Blue Quant/MPN

Made by NISSUI

For fast and easy quantification of coliform bacteria according to MPN (Most Probable Number Method), e.g. according to DIN EN ISO 9308-2:2014-06. Optimised for use with EC Blue 100 and 100P.

### A. Introduction

The unique, innovative design of the EC BlueQuant/MPN allows rapid and reliable processing of MPN tests – simple to operate and without any additional equipment, except an incubator.

The method is based on the standardised MPN method that uses at least three different dilution levels in increments of 10 and 5 compartments per dilution. Testing with the EC BlueQuant/MPN does not require a series of dilutions. When pouring the mixture, due to the special layout, the compartments are filled in such a way as to ensure correct serial dilution. After incubating the sample for 24 hours at about 35 °C, the result can be read off the device and converted to cfu/ml using ISO standard tables (e.g. DIN EN ISO 9308-2:2014-06).

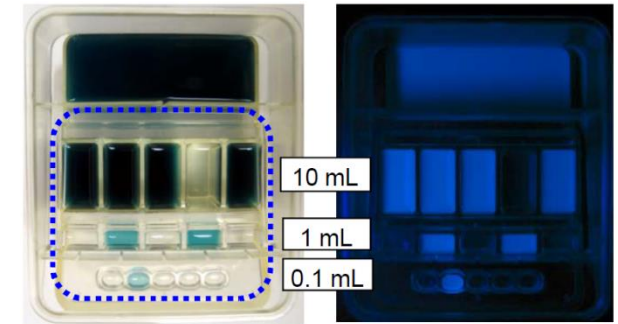
Presence of *E. coli* can easily be detected by the occurrence of fluorescence under UV light (see EC-Blue Mini UV lamp Art. No. KK49.1). Approximately 95 % of all *E. coli* strains produce the enzyme  $\beta$ -glucuronidase, which reacts with an enzyme substrate contained in EC Blue to release a fluorescent dye.

Detection limit: 1 cfu/100 ml for coliforms and *E. coli*.  
The set consists of 18 EC Blue Quant/MPN chambers including lids.

### B. Application

1. EC Blue solution (100 ml) is prepared using a watery sample and EC Blue 100/100P or a 10fold dilution of EC Blue 10 (Art. Nos. 0202.1, 0209.1, 7754.1).
2. The EC Blue solution is poured into the slope (see figure, area is surrounded by a blue broken line).
3. After each row of wells (10, 1, 0.1 ml) is filled, excess of the EC Blue solution is pooled into the upper reservoir.
4. The lid is applied, and then the device is incubated at 35-37 °C for 24 h.
5. After incubation, see if fluorescence is observed in wells by UV irradiation (366 nm), indicating presence of *E. coli* in the sample. A blue color observed in the wells indicates presence of Coliforms in general (see figure).
6. Positive wells may be determined by comparing to a color comparator (positive control, Art. No. 0349).
7. The total cell number is determined by using the MPN calibration scale.

In the figure shown here, the number of positive cells corresponding to rows 10, 1, and 0.1 ml, are 4 (10 mls), 2 (1 ml), and 1 (0.1 ml), respectively. The total colony number is determined as 26 cfu / 100 ml from the MPN calibration scale.



### C. General precautions

1. Read and follow precisely the warning and direction for use described on the package insert and/or label.
2. EC Blue solution is carefully poured into a slope of a MPN plate to keep the solution from overflowing.
3. After incubation, a MPN plate is gently treated to avoid a contamination among each of wells
4. When media touched eyes or mouth, immediately wash with plenty of water, and consult a physician.
5. Manipulations with microorganisms always involve certain risks of laboratory-acquired infections. Manipulations should be done under the supervision of trained specialists with biohazard protection measures.

### D. Disposal of waste

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.

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**EC Blue Quant/MPN**

18 pieces incl. lids 0199.1

## 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		