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<https://www.cellspect.com/>

Significance of measurement

This kit is for measuring the number of *Escherichia coli* (*E. coli*). Currently, the number of *E. coli* is measured by incubating *E. coli* for 24 hours on an agar plate or in agar medium. A clean environment is required to prepare the culture medium, and the analyst's subjective may affect the measurement. The kit provides a simple, rapid, and low-cost method for measuring *E. coli* that overcomes all the shortcomings of existing technologies. In addition, 96 samples can be analyzed simultaneously. This kit is able to measure the number of *E. coli* in all kinds of liquids including sewage, industrial wastewater, river water, groundwater, and drinking water, etc., and quickly detect fecal contamination that causes waterborne diseases and food poisoning.

Measurement principle

The culture medium for *E. coli* contains organic and inorganic compounds that promote *E. coli* growth, as well as a specific fluorogenic enzyme substrate in which a fluorescent molecule is bound to β -D-glucuronide. The specific fluorogenic enzyme substrate does not emit fluorescence until being degraded by β -D-glucuronidase which is specifically produced by *E. coli*. Because the fluorescence intensity is proportional to the amount of degradation of the specific fluorogenic enzyme substrate, the change of fluorescence intensity over time (slope) represents the enzyme activity. The enzyme activity is equal to the amount of enzyme, that is, the number of *E. coli*. The number of coliform bacteria can be measured by using a specific fluorogenic enzyme substrate in which a fluorescent molecule is bound to β -D-galactopyranoside.

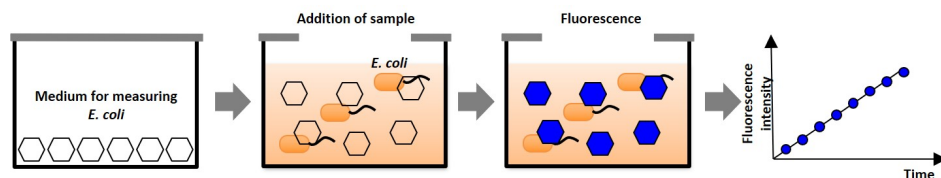


Figure 1 : Process diagram of measurement

Kit contents

96 tests (Product code : RECEL964)

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|--|--------------------|
| 1. The plate with <i>E. coli</i> selective freeze-dried medium | 96-well \times 4 |
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Materials required but not supplied

- Fluorescence microplate reader
- Micropipette and tip
- Tweezers with sharp tips

Assay protocol

Measurement

- (1) Set the plate reader at 37 °C
- (2) Make holes in the seal attached to the plate (or remove the seal.)
- (3) Dispense 200 μ L of the test sample into each well of medium-containing 96-well plate.
- (4) Set the excitation wavelength at 360 nm and the measurement wavelength at 460 nm. Measure every 10 minutes for 3 hrs.

Notes

1. Regarding test samples:
 - Please use fresh samples or refrigerated samples.
 - Mix the sample well and collect it aseptically with caution to prevent contamination.
 - Be careful not to scatter the medium components when adding the sample.
 - There is always the risk of infection when handling microorganisms, so please take biohazard countermeasures under the guidance of technicians.
2. Regarding measurement:
 - Do not expose 96-well plate to light.
 - Do not use a damaged container or a container containing foreign matter.
 - Please do not use expired products as the quality cannot be guaranteed.
 - The bottom surface of plate is immobilized with medium which might be shed by contact with the pipette. Please make sure the pipette does not touch the bottom or wall of the plate.
 - Be sure to bring the plates to room temperature (20~25°C) prior to use.
 - Adjust the gain of the fluorescent plate reader before measuring.
 - If the number of *E. coli* in the sample is small, extend the measurement time.
 - *Escherichia coli* O157: H7 does not produce β -glucuronidase, so it cannot be measured by this assay.
 - Sterilize the used medium, container, equipment, etc. in an autoclave, and then dispose them in accordance with the regulations regarding waste.

Product Specifications

Number of tests : 96 tests \times 4 plates

Measurement method : Enzyme activity assay

Excitation wavelength : 360 nm

Measurement wavelength : 460 nm

Samples : Sewage, industrial wastewater, river water, groundwater, drinking water

Storage temperature : Store at 2-8°C

Expiration date : 6 months after production

References

- 1.) Hisashi Satoh, Kai Kikuchi, Yutaka Katayose, Shu Tsuda, Reiko Hirano, Yuga Hirakata, Masaaki Kitajima, Satoshi Ishii, Mamoru Oshiki, Masashi Hatamoto, Masahiro Takahashi, Satoshi Okabe (2020) Simple and Reliable Enumeration of *Escherichia coli* Concentrations in Wastewater Samples by Measuring β -D-glucuronidase (GUS) Activities via a Microplate Reader. *Science of The Total Environment*. In press
- 2.) Hisashi SATOH, Shu TSUDA Shu, Kai KIKUCHI Kai, Reiko HIRANO Reiko (2019) “Development of a simple and rapid method for determining coliforms in wastewater based on fluorogenic enzymatic substrate” *Journal of Japan Sewage Works Association*. 56(684), 110-117.

Manufacturer and distributor

Cellspect Co., Ltd.

2-4-23 Kitaiioka, Morioka, Iwate 020-0857 Japan

※ QuaResearch is the name of the reagent kit of Cellspect Co., Ltd.

Contact information

Cellspect Co., Ltd.

TEL : 019-134-6616

e-mail : support@cellspect.com

URL : <https://www.cellspect.com/>

- ※ This kit is for research use only. Not for use in diagnostic procedures.
- ※ For the latest information on products such as instruction manuals, measurement protocols, etc., please check the support corner of our website below.
<https://www.cellspect.com/>
- ※ This product is for research use. Please understand that its value cannot be fully guaranteed.
- ※ The indicated performance is a standard value when a general-purpose microplate reader is used. Please understand that variation may occur depending on the types of equipment.
- ※ When making inquiries regarding quality, please confirm the Lot No. attached to the packaging bag of reagent kits and contact us.
- ※ Product specifications, service, packaging form, and measurement protocols may be changed without notice. Please follow this instruction manual properly.
- ※ Please follow the attached Safety Data Sheet (SDS) for transportation, handling, processing, and disposal of this product.