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In Vitro Diagnostic Reagents
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A Test Kit for the Detection of Heat-stable Enterotoxin of *E. coli*

COLIST EIA "SEIKEN"

96 tests

Escherichia coli has long been considered important as a pathogen to cause diarrhea. Renewed attention has been directed at toxigenic *E. coli* in recent years because of the growing threat it poses as a major cause of imported infectious diseases. The development of reagents suitable for use by general laboratories is widely desired for the purpose of its easy identification.

This reagent was developed to detect heat-stable enterotoxin of *E. coli* (ST). Very pure ST prepared by peptide synthesis and monoclonal antibodies are used in this test kit to ensure the strong, highly specific reactions required for ST detection.

FEATURES

1. Assay sensitivity is 10 to 20 ng/mL and the reagent has a good correlation with the conventional biossay using suckling mice. The monoclonal antibody used in the test is capable of detecting both STh (ST produced by human origin *E. coli*) and STp (ST produced by animal origin *E. coli*), but they do not react with LT of *E. coli* nor with enterotoxin produced by so-called NAG-*Vibrio* or *Yersinia*.
2. As this kit uses separate-type microplates, assay can be conducted according to the number of specimens.

CONTENTS

1. **ST-coated microtitration well strips**: 16 wells/strip × 6
   Microtitration wells are coated with synthesized enterotoxin and stabilizer.
2. **Positive control**: 1 mL 1 vial
   Culture fluid of ST positive strain
3. **Negative control**: 1 mL 1 vial
   Culture fluid of ST negative strain
4. **Enzyme-labeled antibody**: 0.1 mL 1 vial
   Anti-ST monoclonal antibody labeled with Peroxidase (POD)
5. **Diluent for enzyme-labeled antibody**: 1.5 mL 1 vial
   Tris-HCl buffer solution containing bovine serum albumin as stabilizer at 1 w/v% concentration.
6. **Substrate A**: Equivalent 4 mL 6 vials
   Lyophilized Citrate-phosphate buffer saline containing O-phenylenediamine 3.3 mg per mL.
7. **Substrate B**: 30 mL 1 vial
   Citrate-phosphate buffer solution containing 0.02 vol% Hydrogenperoxide.
8. **Stock wash solution (×10 conc.)**: 60 mL 1 vial
   Phosphate-buffered saline containing 0.5 vol% Tween 20.
9. **Stopping solution**: 12 mL 1 vial
   0.75mol/L Sulfuric acid
10. **Plate holder**: 1 pc.

INTENDED USE

Detection of heat-stable enterotoxin (ST) of *E. coli* originated in digestive tract.

PRINCIPLE OF MEASUREMENT

Specimens (Culture fluid of *E. coli*) are dispensed into the microtitration wells coated with the synthesized ST. Then, the anti-ST monoclonal antibody labeled with peroxidase enzyme are added to the wells to allow ST in the specimen to compete with surface-bound St for enzyme-labeled antibody in the formation of immune-complex. Unbound complex is rinsed away and surface-bound peroxidase is detected by the ensuing tracer reactions using hydrogen-peroxide as substrate and O-phenylenediamine as color reagent.

The ST in the specimen, if amply present, will exclusively combine with enzyme-labeled antibody leaving hardly any antibody free to combine with the surface-bound ST. Hence there will be no color formation in tracer reactions. In the case of specimens containing no or very little ST, enzyme-labeled antibody will be trapped, on the contrary, by the surface-bound ST resulting in color formation in the tracer reactions.

PROCEDURES

1. Materials and reagents

1) Material required but not supplied
   Test tube, centrifugation tubes, or small test tubes
   Mixer for microplates
   Micropipette 10, 100 and 200 μL
   Spectrophotometer for microplates
2) Reagent: COLIST EIA "SEIKEN" 1 kit

2. Preparation of the reagents

Reagent	Method of preparation	Prepared reagent	Shelf life after preparation
Enzyme-labeled antibody (× 10 conc.)	Add 1mL of wash buffer to the vial and shake it well.	Enzyme-labeled antibody solution	1 month when stored at 2-10℃
Stock wash solution	A proper volume for immediate requirements is taken and diluted.	Wash buffer	1 month when stored at 2-10℃
Substrate A Substrate B	Just before use, dissolve Substrate A by adding 4 mL of Substrate B to each vial of Substrate A.	Substrate solution	Use within 1 hour after preparation

3. Test procedures

1) Add approximately 2 mL of CA-YE broth to a test tube, and inoculate a small volume of test strain of *E.coli* into it.
2) Soon after inoculation, shake-culture the strain vigorously at 37℃ for 18 hours (overnight).
3) Transfer the culture fluid to a centrifugation tube or a small test tube and centrifuge it at minimum 3000 rpm for 30 minutes to precipitate the cells.
4) Insert microtitration-well strips to the holding frame right before use, and discard well contents. Then add wash buffer to the wells, and rinse them once.
   Note) After rinsing, avoid drying and use the wells within the same day.

5) Add specimen and controls as shown below.
Test example:

(B)	(P)	(N)	(S)	(S)	(S)	(S)	(S)
(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)

(B) : Add nothing to the blank well.
(P) : Add 200 μL of positive control.
(N) : Add 200 μL of negative control.
(S) : Add 200 μL of specimens. (Each specimens uses one microtitration well.)

6) Add 10 μL of enzyme-labeled antibody solution to each well, except the blank well, mix by micromixer for 1 minute, and leave the plate undisturbed at room temperature for 90 minutes.
7) Aspirate the well contents.
8) Add approximately 200 μL of wash buffer to each well, and mix with micromixer for 30 seconds, and aspirate the well contents again. Repeat washing procedures a further four times. After washing, discard the well contents by inverting the plate, and place the plate inverted on a paper towel and tap it gently to remove thoroughly the residual solutions from the wells.
9) Add 100 μL of substrate solution to all of the wells including blank wells.
10) After mixing by micromixer for several seconds, leave the plate undisturbed at room temperature for 30 minutes in the dark.
11) Expeditiously add 100 μL of stop solution to each well.

☆ CA-YE broth composition:

Casamino acid	2 w/v%
Yeast extract	0.6 w/v%
NaCl	0.25 w/v%
K_2HPO_4	0.871 w/v%
Salts solution*1	0.1 vol%

Before use, pH is adjusted to 8.0- 8.2, and the solution is sterilized by autoclaving it at 120℃ for 10 minutes.

*1 Composition of salts solution :

$MgSO_4$	5 w/v%
$MnCl_2$	0.5 w/v%
$FeCl_3$	0.5 w/v%

These are dissolved in 0.0005 mol/L H_2SO_4.

READING

Results are read by the naked eye.

1. Compare the colors produced by specimens with those by negative and positive controls. Specimens which produced colors as distinct as negative control are regarded as negative.
2. Specimens whose wells remain, after the test, as transparent as those of positive control are regarded as positive.
3. Specimens which produced confusing results, difficult to read visually, should be subjected to OD measurement, adjusting the photometer to zero against blank wells.

$$\text{Positive} = \frac{\text{(OD of the specimen)}}{\text{(OD of negative control)} - \text{(OD of positive control)}} \leqq 0.5$$

OD of the negative specimens will, in this test, be approximately 1.0 while that of positive ones will be lower than 0.2.

PERFORMANCES OF THE TEST

1. **Sensitivity**
The sensitivity of the test is 10 - 20 ng/mL when the ST with known values are used.
2. **Specificity**
The reagent can detect heat-stable enterotoxin of *E. coli* specifically but does not react with heat-liable enterotoxin of *E. coli* or heat-stable enterotoxin of *Yersinia* or NAG-*vibrio*.
3. **Correlation**
A more than 99% correlation was found to exist between this test and conventional bioassay using suckling mice.

CAUTION

1. The ST producibility of *E. coli* varies according to colonies from which the test specimen is prepared, so it is recommended to test a strain by selecting multiple colonies.
2. ST produced in culture fluid will lose its activity as time elapses, so the test should use fresh specimens soon after culture.
3. Substances which influence the activity of Peroxidase, such as sodium azide or formalin shall not be used in the test.
4. Specimens with turbidity because of insufficient centrifugation or those with high viscosity by cell lysis will give suppressed color formation.
5. Although ST activity will survive 100℃ for 15 minutes heating, it is not advisable to use heated specimens in this test.
6. Culture supernatant may have been contaminated with live cells, so wash buffer after use shall be sterilized by autoclaving at 121℃ for 20 minutes. Instruments used for the test should be disposed of after soaking in 0.1 w/v% sodium hypochlorite solution (available chlorine approximately 1000 ppm) for 1 hour or more, or after autoclaving as shown above.
7. Do not combine or mix reagents from different manufacturing lots.

STORAGE AND SHELF LIFE

Storage: 2 -10℃
Shelf life : Up to the expiry date on the label.

PACKAGE

COLIST EIA "SEIKEN" 96 tests 1 box

REFERENCES

1. Haruo Ikemura: Synthesis of a Heat-stable Enterotoxin (STh) Produced by a Human Strain SK-1 of Enterotoxigenic *Escherichia coli*, Bull. Chem. Soc. Jan., **57**, 2543 (1984).
2. Michael R. Thompson: Simple and Reliable Enzyme-Linked Immunosorbent Assay with Monoclonal Antibodies for Detection of *Escherichia coli* Heat-Stable Enterotoxins, J. Cline. Microbio., **20**, 59 (1984).

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