

S&A REAGENTS LAB LTD.,PART.

4 Soi Latphraowanghin 28 , Latphrao-wanghin Rd.,
Lat Phrao, Bangkok 10230, Thailand.



Tel. (+662) 538-8622
Fax. (+662) 931-6098
e-mail : saplab1990@gmail.com
www.serotest-thailand.com



***Escherichia coli* Antisera**

E. coli antisera are produced for serological identification of *E. coli*, based on agglutination method. These polyclonal antibodies are prepared by immunizing rabbit with the standard strains.

For high specificity; the non-specific agglutinins have been removed by absorption.

Preservative : 0.1% Sodium azide

Storage condition : 2-8°C

Do not freeze the antisera.

Complete identifications of *E. coli* requires cultural isolation, biochemical characterization and serotyping. However well defined the serology, the use of serological procedures do not supersede cultural isolation and biochemical characterization.

O-antigen testing

A. Slide agglutination of live organisms

1. Place 1-2 drops of saline onto control area and place a drop of polyvalent antiserum onto test area of clean glass slide.
2. Using platinum wire, transfer a portion of a loopful of growth from TSI slant onto the drop of saline and antiserum, mix the cultures and serum or cultures and saline well. Tilt the glass slide back and forth for one minute. If there is clumping in the control, the culture is rough and serological tests cannot be interpreted on slide testing.
3. If the live organisms doesn't give positive reaction with antiserum, mix 2 loops of the culture with 1 ml. of saline then heat at 100°C for 1 hr. then repeat step 1 and 2.
4. Further testing of the isolate should be conducted as described in steps 1-3 with monovalent O antisera to reveal the full O antigenic grouping of the isolate. Always confirm the O grouping by slide agglutination on heat killed organisms, see next step.

B. Slide agglutination of heat killed organisms

If the live organisms give positive reaction with antiserum, mix 2 loops of the culture with 1 ml. of saline then heat at 100°C for 1hr. then repeat the agglutination test as above using monovalent O antiserum on the heated cell suspension. This should be done to identify the O antigen type as distinct from the K antigen.

H-antigen Testing

1. Allow the organism to pass through semi-solid medium. Then inoculate the resulting organism into 2 ml of Tryptic soy broth tubes and incubate at 37°C for 6-8 hrs.
2. After incubation period, add 2 ml. of 0.85% saline containing 1% (v/v) Formalin, allow the inoculum at the room temperature for 30 minutes. Transfer the 0.45-0.5 ml of inoculum into duplicate of 12x75 mm tube.
3. For the first tube, add 2 drops of *E. coli* H7 antiserum (AS546) and not add any antiserum for another tube for Negative control. Allow both tube to stand in water bath at 48-50 °C for 1 hour.
4. After incubation period, observe the result by checking the negative control tube which should shown an even suspension; clumping in the control indicates that the culture is rough and serological tests cannot be interpreted, then observe another tube for the flocculation reaction; that is Positive result. An isolate producing a distinct positive reaction is assumed to be an *E. coli* bearing the H antigenic factors represented by that antiserum.

***E. coli* Polyvalent Antisera**

Cat. No.	Description	Specific Factors
AS521	E. coli (O&K) Polyvalent I	O25 : K11
		O26 : K60
		O44 : K74
		O55 : K59
		O78 : K80
		O111 : K58
		O114 : K-
		O119 : K69
AS530	E. coli (O&K) Polyvalent II	O86 : K61
		O124 : K72
		O125 : K70
		O126 : K71
		O127 : K63
		O128 : K67
AS537	E. coli (O&K) Polyvalent III	O18a O18c : K77
		O20a O20b : K84
		O28 : K73
		O112a O112c : K66

***E. coli* Monovalent Antisera**

Cat. No.	Description	Cat. No.	Description
AS522	E. coli O25 : K11	AS534	E. coli O126 : K71
AS523	E. coli O26 : K60	AS535	E. coli O127 : K63
AS524	E. coli O44 : K74	AS536	E. coli O128 : K67
AS525	E. coli O55 : K59	AS538	E. coli O18a O18c : K77
AS526	E. coli O78 : K80	AS539	E. coli O20a O20b : K84
AS527	E. coli O111 : K58	AS540	E. coli O28 : K73
AS528	E. coli O114 : K-	AS541	E. coli O112a O112c : K66
AS529	E. coli O119 : K69	AS545	E. coli O157
AS531	E. coli O86 : K61	AS546	E. coli H7
AS532	E. coli O124 : K72	AS547	E. coli O1 : K1
AS533	E. coli O125 : K70	AS548	E. coli O2

Reference:

1. Kauffmann, F ., Classification of Bacteria
2. Edward, P.R. and Ewing, W.H. , Identification of of Enterobacteriaceae , Fourth Edition, Burgess Company, Minnesota 1986

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