

Rapidcult™ E. coli

Culture medium for enrichment of pathogenic E.coli

General Information

Medium for the fast enrichment of pathogenic *E. coli* from meat products.

Intended Use

Rapidcult *E. coli* is a selective enrichment broth for the fast detection of pathogenic *E. coli* in meat products.

Rapidcult *E. coli* is intended for use in microbiology laboratories analyzing food.

Microbiological examinations are to be performed by trained staff only.

Mode of Action

The interaction of selected peptones, phosphate buffer, carbohydrates and mineral salts promotes rapid growth of pathogenic *E. coli*.

This medium enables the detection of *E. coli* in food matrices such as minced meat and beef trims.

Typical composition (g/Liter)

Peptones 19.0; Phosphate Buffer 5.0; Carbohydrates 5.0; Sodium thioglycolate 0.1; Mineral Salts 0.1.

Preparation

For 1500 ml - typically used for 375 g food samples:

Suspend 43.8 g of dehydrated Rapidcult *E. coli* in 1500 ml of pre-warmed (42°C) sterile, demineralized water and mix gently until completely solved.

For 225 ml - typically used for 25 g food samples:

Suspend 6.6 g of dehydrated Rapidcult *E. coli* in 225 ml of pre-warmed (42°C) sterile, demineralized water and mix gently until completely solved.

pH: 7.0 ± 0.2 at 25 °C.

The broth is clear and brownish in color.

Store the prepared broth at 42°C until use (not longer than 3 hours).

Experimental Procedure

Prepare food samples using local standard methods and guidelines.

Enrichment:

Each sample is homogenized in pre-warmed (42°C) Rapidcult *E. coli* enrichment broth and incubated for 8 - 22 hours at 42 °C.

Detailed information about incubation can be found in the protocol of the chosen detection method.

Detection:

The cultural based detection is performed by using immunomagnetic separation (IMS) with antibody coated magnetic beads.

Therefore 1 ml per sample of incubated enrichment broth is mixed with bead suspension. After two washing steps the bead suspension is streaked out onto CT-SMAC agar plates. CT-SMAC agar plates are incubated for 24 ± 2 h at 35-37 °C.

After incubation of inoculated CT-SMAC agar, plates are examined for presumptive colonies. Confirmation is done by using SinglePath *E. coli* O157 or foodproof® *E. coli* O157 Detection Kit. Screening of the colonies for Shiga Toxin production is checked using Dupath Verotoxins test.

The alternative antibody based detection is done by using Singlepath® *E. coli* O157. 150-160 µl of the enrichment sample is applied to the circular sample port of the test device. When *E. coli* O157 are present in the sample, the formation of 2 distinct red lines is observed after 20 minutes. Only 1 red line (Control) is observed when no *E. coli* O157 are present.

The alternative nucleic acid based detection method is done by real-time Polymerase Chain Reaction (PCR). The isolation of DNA is performed by using the foodproof® StarPrep One Kit followed by the foodproof® *E. coli* O157 Detection Kit for detection of *E. coli* O157 specific DNA.

The protocol for detection of *E. coli* O157 from other sample types using 25 g is comparable to the procedure described above. For further details see application note.

External Validation Study

The study compared alternative methods using the novel RapidCult™ *E. coli* enrichment to the USDA/FSIS reference method (MLG 5.04) for the detection of *E. coli* O157 from meat products.

The validation study was performed with pooled ground beef and beef trim samples (375 g) following recommendation of the USDA on the number of sample types and the statistical analysis. In total 60 ground beef and beef trim samples, respectively, were artificially spiked in order to obtain fractional positive samples.

For enrichment of alternative methods, 43.8 g of dehydrated RapidCult™ *E. coli* Enrichment Broth was dissolved in 1.5 liters of pre-warmed (42 °C), sterilized, deionized water, stored in water bath for maximum two hours at 42 °C and added to 375g of each ground beef or beef trim sample. Samples were placed in stomacher bags. Mixing was conducted manually. Incubation was conducted for 8 h to 22 h at 42 ± 2 °C statically.

Samples were taken at several time points. At all sampling points the bags were mixed manually by back and forth panning. Aliquots of 10 ml of enrichment suspension were kept in sterile glass test tubes for further processing.

The RapidCult™ *E. coli* enrichment of pooled samples was followed by cultural, immunological and real-time PCR based methods to detect *E. coli* O157:H7. The cultural based detection was performed by using IMS and Merck CT-SMAC agar plates. As an alternative antibody based method Singlepath® *E. coli* O157 was used.

The combination of foodproof® StarPrep One Kit and foodproof® *E. coli* O157 Detection Kit was used for the alternative real-time PCR based method.

The reference method was based on the MLG 5.04 "Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products" in USDA/FSIS Microbiology Laboratory Guidebook, Rev #4, 1/28/08 using 65g of sample. Bags were incubated for 22h at 42 +/- 2°C. All media and reagents needed were prepared according to the manufacturers instructions.

The method comparison study showed that the alternative methods used performed equal to or better than the USDA MLG 5.04 reference method. In addition the detection time of *E. coli* O157 could be reduced significantly by the combination of RapidCult™ *E. coli* and Singlepath® *E. coli* O157 and RapidCult™ *E. coli* and foodproof® *E. coli* O157 Detection Kit, respectively

The Singlepath® *E. coli* O157 test enables the detection of one *E. coli* O157 per 375g of ground beef after 12h enrichment and in beef trim after 8 h enrichment using the novel Rapidcult *E. coli* Enrichment broth.

The foodproof® *E. coli* O157 Kit enables the detection of one *E. coli* O157 per 375g of ground beef after 10h enrichment and in beef trim after 8h enrichment using the novel Rapidcult *E. coli*.

The validation is consistent with FSIS guidance. Questions about the use of the Merck test kit by USDA-FSIS regulated facilities should be directed to AskFSIS (<http://askfsis.custhelp.com/>).

Literatur

- M1.Microbiology Laboratory Guidebook (MLG 5.04): Detection, Isolation and Identification of *E. coli* O157:H7 from meat products. (http://www.fsis.usda.gov/science/Microbiological_Lab_Guidebook/index.asp MLG 5.04 as accessed on 01/28/2008.
- Application Note

Ordering information

Rapid detection with foodproof® RT-PCR

Enrichment of *E.coli*

Product	Ordering No.	Pack size
Rapidcult™ <i>E. coli</i> Culture Medium for enrichment of pathogenic <i>E. coli</i>	1.00026.0100	100 g
Rapidcult™ <i>E. coli</i> Culture Medium for enrichment of pathogenic <i>E. coli</i>	1.00026.0500	500 g

Sample preparation

Product	Ordering No.	Pack size
ShortPrep I Kit	1.20471.00001	96 tests
ShortPrep II Kit	1.20470.00001	96 tests

RT-PCR

Product	Ordering No.	Pack size
Foodproof® <i>E.coli</i> O157 5'Nuclease	1.20455.00001	96 tests
Foodproof® <i>E. coli</i> O157 Hybridization probes (LC1.x, 2.0)	1.20466.00001	96 tests

Rapid Screening of *E. coli* O157 with Singlepath®

Enrichment

Product	Ordering No.	Pack size
Rapidcult™ <i>E. coli</i> Culture Medium for enrichment of pathogenic <i>E. coli</i>	1.00026.0100	100 g
Rapidcult™ <i>E. coli</i> Culture Medium for enrichment of pathogenic <i>E. coli</i>	1.00026.0500	500 g
Singlepath® <i>E. coli</i> O157	1.04141.0001	25 tests

Classical culture method for *E.coli* O157

Enrichment of *E. coli*

Product	Ordering No.	Pack size
Rapidcult™ <i>E. coli</i> Culture Medium for enrichment of pathogenic <i>E. coli</i>	1.00026.0100	100 g
Rapidcult™ <i>E. coli</i> Culture Medium for enrichment of pathogenic <i>E. coli</i>	1.00026.0500	500 g

Detection of *E. coli* O157

Product	Ordering No.	Pack size
Sorbitol-MacConkey Agar (SMA Agar)	1.09207.0500	500 g
CT-Supplement	1.09202.0010	10 vials

Confirmation

Product	Ordering No.	Pack size
Fluorocult® <i>E. coli</i> O157:H7 Agar	1.04036.0500	500 g
UV-Lamp	1.13203.0001	1 piece
Singlepath® <i>E. coli</i> O157	1.04141.0001	25 tests

Besides the enrichment of E. coli O157 the medium can be used for the testing of Verotoxins producing E. coli like O104 out of several sample material.

Classical microbiological method

Enrichment

Product	Ordering No.	Pack size
Rapidcult™ E. coli Culture Medium for enrichment of pathogenic E. coli	1.00026.0100	100 g
Rapidcult™ E. coli Culture Medium for enrichment of pathogenic E. coli	1.00026.0500	500 g

Detection

Product	Ordering No.	Pack size
Chromocult® coliform Agar	1.10426.0500	500 g
Chromocult® TBX Agar	1.16122.0500	500 g

Confirmation

Post enrichment

Product	Ordering No.	Pack size
CAYE-Broth	1.00060.0100	100 g
CAYE Broth Supplement	1.00051.0010	10 vials
Polymyxin B sulfate Bacillus cereus Selective Supplement	1.09875.0010	10 vials

Quality Control

Control Strains	Inoculum (cfu/250 ml)	Growth after 8 hours (cfu/ml)	Reference Media
E. coli O157 ATCC 43889	10-100	$\geq 10^6$	Tryptic Soy Agar (TSA)
E. coli O157 ATCC 43890	10-100	$\geq 10^6$	Tryptic Soy Agar (TSA)
E. coli O157 ATCC 43894	10-100	$\geq 10^6$	Tryptic Soy Agar (TSA)
E. coli ATCC 11775	$> 10^4$	No limit	Tryptic Soy Agar (TSA)
Staphylococcus aureus ATCC 25923	$> 10^4$	No growth	Tryptic Soy Agar (TSA)

Product	Ordering No.	Pack size
Duopath® Verotoxins	1.04144.0001	25 tests

Rapid method for screening with Duopath® Verotoxins

Enrichment of E. coli

Product	Ordering No.	Pack size
Rapidcult™ E. coli Culture Medium for enrichment of pathogenic E. coli	1.00026.0100	100 g
Rapidcult™ E. coli Culture Medium for enrichment of pathogenic E. coli	1.00026.0500	500 g

Post enrichment

Product	Ordering No.	Pack size
CAYE-Broth	1.00060.0100	100 g
CAYE Broth Supplement	1.00051.0010	10 vials
Polymyxin B sulfate Bacillus cereus Selective Supplement	1.09875.0010	10 vials

Screening

Product	Ordering No.	Pack size
Duopath® Verotoxins	1.04144.0001	25 tests

Rapidcult™ E. coli Protocol for 375 g ground beef using foodproof® E. coli Detection kit

Dissolve 43.8 g of dehydrated Rapidcult E. coli medium in 1.5 liters of pre-warmed (42°C), sterilized, deionized water



Add 375 g of beef trim to pre-warmed (42°C) medium and homogenize



Incubate **10** hours at 42°C



Extract DNA from enriched sample using the **foodproof® StarPrep One** or **ShortPrep II Kit** Protocol



Conduct RT-PCR analysis using the **foodproof® E. coli O157 Detection Kit** Protocol

Rapidcult™ E. coli Protocol for 375 g ground beef using Singlepath® E. coli O157

Dissolve 43.8 g of dehydrated Rapidcult E. coli medium in 1.5 liters of pre-warmed (42°C), sterilized, deionized water



Add 375 g of beef trim to pre-warmed (42°C) medium and homogenize



Incubate **12** hours at 42°C



Dispense 160 µl of the enriched sample into the sample port on **Singlepath® E.coli O157**



Read result after 20 minutes

Rapidcult™ E. coli Protocol for 375 g ground beef using classical microbiological methods

Dissolve 43.8 g of dehydrated Rapidcult E. coli medium in 1.5 liters of pre-warmed (42°C), sterilized, deionized water



Add 375 g of beef trim to pre-warmed (42°C) medium and homogenize



Incubate **10** hours at 42°C



Prepare immunoconcentrated solution of E. coli O157 by IMS



Streak onto CT-SMAC



Incubate 24 hours at 35 – 37°C



Confirm presumptive colonies

Rapidcult™ E. coli Protocol for 375 g beef trim using foodproof® E. coli Detection kit

Dissolve 43.8 g of dehydrated Rapidcult E. coli medium in 1.5 liters of pre-warmed (42°C), sterilized, deionized water



Add 375 g of beef trim to pre-warmed (42°C) medium and homogenize



Incubate **8** hours at 42°C



Extract DNA from enriched sample using the **foodproof® StarPrep One** or **ShortPrep II Kit** Protocol



Conduct RT-PCR analysis using the **foodproof® E. coli O157 Detection Kit** Protocol

Rapidcult™ E. coli Protocol for 375 g beef trim using Singlepath E. coli O157

Dissolve 43.8 g of dehydrated Rapidcult E. coli medium in 1.5 liters of pre-warmed (42°C), sterilized, deionized water



Add 375 g of beef trim to pre-warmed (42°C) medium and homogenize



Incubate **8** hours at 42°C



Dispense 160 µl of the enriched sample into the sample port on **Singlepath® E.coli O157**



Read result after 20 minutes

Rapidcult™ E. coli Protocol for 375 g beef trim using classical microbiological methods

Dissolve 43.8 g of dehydrated Rapidcult E. coli medium in 1.5 liters of pre-warmed (42°C), sterilized, deionized water



Add 375 g of beef trim to pre-warmed (42°C) medium and homogenize



Incubate **8** hours at 42°C



Prepare immunoconcentrated solution of E. coli O157 by IMS



Streak onto CT-SMAC



Incubate 24 hours at 35 – 37°C



Confirm presumptive colonies

Rapidcult™ E. coli Protocol for 25 g samples

