REF AEB520020 / AEB620027
REF AEB184135 / AEB184135/10 /AEB150022

620027 – **E** - en - 2015/12 EN

REBECCA™

For microbiological control only

Selective medium for the enumeration of *Escherichia coli* β-D- glucuronidase positive and Enterobacteriaceae (non *E. coli*) in food products.

Certificate No: AES 10/06-01/08
Method REBECCA™ base or REBECCA™ + EB (Enumeration of *E. coli*)
ALTERNATIVE METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
http://nf-validation.afnor.org/en
For food and animal feeding stuffs
The date of end of validity for the NF VALIDATION certification is indicated on the certificate

Certificate No: AES 10/07-01/08
Method REBECCA™ + EB (Enumeration of Enterobacteriaceae)
ALTERNATIVE METHODS FOR AGRIBUSINESS
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SUMMARY AND EXPLANATION

REBECCA™ is a chromogenic medium for the direct enumeration without confirmation in products for human and animal consumptions of:

- *E. coli* β- D-glucuronidase positive (1),
- *E. coli* β- D-glucuronidase positive and of enterobacteria (1,2)

PRINCIPLE

The enumeration of *E. coli* is done by the detection of β- D-glucuronidase colouring the colonies in blue with or without blue halo.

The screening of others enterobacteria (non *E. coli*) is done by the addition to REBECCA™ base of a specific supplement that colours the colonies in pink to red.

The mixture of selective agents inhibits the growth of the interfering flora.

CONTENT OF KIT

Ready to use media	
AEB520020	Pack of 20 plates 90 mm
AEB620027	6 Bottles of 200 mL
REBE*	
REBECCA™ SUPPLEMENT EB	
AEB184135(i)	1 QS 1.2 L
AEB184135/10(i)	10 QS of 1.2L
Dehydrated media REBECCA™ (Base)	
AEB150022:	500g

(*) : Printed on the container

COMPOSITION

Theoretical formula in grams per litre

This medium can be adjusted and/or supplemented according to the performance criteria required:

Peptones and extracts	18.00
Selective compounds	5.25
Chromogenic mix	0.25
Agar	14.00
Mix of selective compounds and chromogenic substrates [a]	

pH final : 7.3

a : MIX in REBECCA™ + EB plates or after addition of REBECCA™ EB supplement (AEB184135 or AEB184135/10)

(i) Reagents containing a substance at a concentration considered dangerous: Vancomycin (<4%)..

SIGNAL WORD : **WARNING**

H317
P261 / P280 / P302 + P352 / P333 + P313

Hazard statement:
H317 : May cause an allergic skin reaction

Precautionary statement:
P261 : Avoid breathing dust/fume/gas/mist/vapours/spray.
P280 : Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 : IF ON SKIN: Wash with plenty of soap and water.
P333+P313 : If skin irritation or rash occurs: Get medical advice/attention.

For further information, refer to the Material Safety Data Sheet.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Material

- Bacteriology incubator.
- Water bath
- Boiling water bath
- Autoclave
- Sterile Petri or aseptic Petri plates
- Bottles resistant to autoclave treatment

Reagents

- Buffered peptone water (ex : 42043)
- Peptone salt (ex : AEB111499 – AEB611498)

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- Comply with Good Laboratory Practice (for example, refer to EN ISO 7218 standard (3))
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to"CLSI® M29-A, Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline– current revision." For further information on

handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH Latest edition, or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents past the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use contaminated plates, or plates that exude moisture.
- Do not use bottles or tubes which show signs of contamination.
- Before use, make sure the tamper-proof seal on the bottle screw caps is intact.
- For dry media, do not use media which are not homogeneous (presence of lumps).
- The medium should be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STOCKAGE CONDITIONS

- Store the plates/Bottles in their box at 2-8°C until the expiry date.
- Store dry media bottles at 1-30°C until the expiry date. Preserve from humidity.
- Plates prepared from the dry medium or ready to use agar in bottles can be kept 15 days at 2 - 8°C.
- Bottles of REBECCA™ base prepared from the dry medium can be kept for 1 month at 2 - 8°C.
- Once rehydrated, the REBECCA™ EB supplement can be kept 24 hours at 2-8°C, or 7 days if frozen (aliquots). The supplement must not undergo more than one cycle of freezing / thawing.
- After incubation, for the modes of storage of the plates, refer to EN ISO 7218 standard.
- AEB184135 – AEB184135/10 – AEB520020 : Protect from light.

SPECIMENS

Follow the recommendations in the current standards to perform specimen collection and preparation.

PREPARATION

- **Dehydrated :**

Suspend 45 g of powder in 1.2 L of purified water. Heat to boiling under constant agitation until complete dissolution of the agar. Autoclave at 121°C for 15 minutes. Cool to 44-47°C. Add aseptically REBECCA™ EB supplement (ex :AEB184135) prepared with 6 ml of a sterile water/Ethanol solution (1:1). Homogenise then dispatch in sterile Petri plates.

- **Ready-to-use bottles:**

Regenerate REBECCA base bottles at 100°C until perfect liquefaction. Cool to 44 - 47°C.

Application 1 : Enumeration of *E. coli* β- D-glucuronidase positive: No supplement is necessary.

Application 2: Simultaneous enumeration of *E. coli* β-D-glucuronidase positive and Enterobacteria (REBECCA™ supplement EB):

Prepare the supplement with 6 ml of a solution water/Ethanol (1:1) and vortex until completely dissolved. Add 1 ml of supplement per 200 ml base bottles. Homogenize gently.

INSTRUCTIONS FOR USE (also refer to appendix I, II and III)

Whatever the claim of REBECCA™ medium, several methods can be put into practice. In all the cases, to use only one plate per dilution:

a) Pour-plate method:

1 ml of the sample or its decimal dilutions in single layer, approximately in 15ml of medium (∅90 mm plates).

b) Surface inoculation:

- by spreading out: of 0.1 ml of the sample and/or its decimal dilutions if necessary.

As to raise the accuracy of the enumeration, it is possible to inoculate 1 ml split onto 3 Ø90 mm plates or 1 Ø140 mm plate (refer to EN ISO 7218 standard (3))

- by automated spreading using spiral (up to 200 µl). (Outside the scope of NF VALIDATION)

Incubate the plates 24 hours ± 2 hours at 37°C ±1°C.

In all the cases, take into consideration only plates containing less than 150 typical colonies. Refer to reading and interpretation chart (Annex 1) for analysis

Note : For pour-plate method, if one suspects a strong contamination of the matrix by micro-organisms likely to invade the agar 's surface, it can be necessary, to facilitate the reading, to pour a double layer (approximately 5 ml) of the same medium maintained in a water bath at 44-47°C.

RESULTS

After incubation, *E. coli* grow as blue colonies with or without halo. Enterobacteriaceae (non *E. coli*) grow as pink to red colonies.
In all cases, retain the plates containing less than 150 characteristic colonies. For the reading and interpretation of results see Annex 1. Refer to EN ISO 7218 standard for calculations and expression of results.

LIMITATIONS OF THE METHOD

- After regeneration, REBECCA™ SUPPLEMENT EB can show the presence of a slight precipitate with no consequence on the performances of the product.
- REBECCA™ base not supplemented, once liquefied can be kept in a water bath for 7 hours at 44-47°C before being used. They can also undergo up to two cycles of melting procedures.
- Ideally the base should be supplemented just before pouring nevertheless it is possible to keep the complete medium (base + supplement) up to 2 hours at 44-47°C. A supplemented REBECCA™ bottle cannot undergo a melting procedure.
- There are a few strains of *E. coli* β-D-glucuronidase negative, for example: *E. coli* O157. These strains will give colonies not typical of the *E. coli* β-D-glucuronidase positive.
- Some strains, other than *E. coli* have a β-D-glucuronidase enzyme and therefore could grow as blue colonies. For example: *Salmonella*…

QUALITY CONTROL

REBECCA™ has been designed and developed to meet the strictest quality requirements.
The results of the strains tested in the batch by batch quality control are given on the quality control certificate available on the Technical Library that can be accessed via our corporate website (www.biomerieux.com).

WASTE DISPOSAL

Unused hazardous reagents must be disposed of following procedures for hazardous chemical waste.
Unused non hazardous reagents may be considered as non hazardous waste and disposed of accordingly. Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. NF ISO 16649-2 : Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of b-glucuronidase-positive Escherichia coli – Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl b-D-glucuronatede. (2001).
2. NF ISO 21528-2 : Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count method (2004).
3. EN ISO 7218 : Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Protect from light
 XXX/Y	Quality control certificate Batch conform to current Quality Control Protocol
	Keep dry
	Date of manufacture

WARRANTY

bioMérieux disclaims all warranties, express or implied, including any implied warranties of MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. bioMérieux shall not be liable for any incidental or consequential damages. IN NO EVENT SHALL BIOMERIEUX'S LIABLITY TO CUSTOMER UNDER ANY CLAIM EXCEED A REFUND OF THE AMOUNT PAID TO BIOMERIEUX FOR THE PRODUCT OR SERVICE WHICH IS THE SUBJECT OF THE CLAIM.

REVISION HISTORY

Changes type categories:

N/A	Not Applicable (first publication)
Correction	Correction of document anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

***Note**: minor typographical, grammar and formatting changes are not included in the revision history.*

Release date	Part number	Change type	Change summary
2015/05	620027D	Administrative	Creation of revision history
		Technical change	Content of kit, Composition
2015/12	620027E	Technical change	Content of kit, Instructions for use

BIOMÉRIEUX

bioMérieux SA
376 Chemin de l'Orme
69280 Marcy-l'Etoile - France

673 620 399 R.C.S LYON
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

Procedure and interpretation guide

ALTERNATIVE METHODS FOR AGRIBUSINESS http://nf-validation.afnor.org/en	**Enumeration of *E. coli* β-D-glucuronidase positive** **Certificate n°: AES 10/06-01/08**	**Simultaneous enumeration of *E. coli* β-D-glucuronidase positive and enterobacteria (non *E. coli*)** **Certificate N° AES 10/06-01/08** **Certificate AES 10/07-01/08**
BASE MEDIUM	**REBECCA™ Base**	**REBECCA™ Base***
SUPPLEMENT TO ADD TO REBECCA™ Base	None	REBECCA™ EB supplement*
INOCULATION	See technical data sheet	See technical data sheet
INCUBATION	24 hours ± 2 hours at 37°C ± 1°C	24 hours ± 2 hours at 37°C ± 1°C
INTERPRETATION	***E. coli* β-D-glucuronidase + :** blue colonies with or without halo	***E. coli* β-D-glucuronidase +:** blue colonies with or without halo **Enterobacteriaceae (non *E. coli* β-D-glucuronidase +) :** pink to red colonies
ENUMERATION (Method of calculation : refer to EN ISO 7218 standard)	***E. coli* β-D-glucuronidase + =** Σ blue colonies	***E. coli* β-D-glucuronidase + =** Σ blue colonies **Enterobacteriaceae =** Σ blue colonies + Σ pink to red colonies

REBECCA base + EB supplement exists in ready-to-use plates for enumeration by surface method (see technical data sheet).

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69280 Marcy-l'Etoile - France

673 620 399 R.C.S LYON
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

Enumeration of Escherichia coli β-D-glucuronidase positive

REBECCA™ Base or REBECCA™ + EB methods

Dilution

X g or X ml of sample + 9 X ml of Buffered Peptone Water or Peptone Salt
or refer to EN ISO 6887 standard for the specific preparations
Carry out successive dilutions if necessary

INOCULATION BY SURFACE METHOD

Spreading of **0.1** ml* onto 1 plate of

REBECCA™ base (AEB620027 – 6 flasks of 200mL)

or **REBECCA™ + EB (AEB520020 – 20 plates Ø 90mm)**
previously dried in an incubator

To increase the accuracy, possibility to spread 1 ml* onto 3 plates Ø 90 mm (see EN ISO 7218 standard).

Incubation for 24 ± 2 hours at 37°C

INOCULATION BY POUR-PLATE METHOD

Inoculate **1 ml*** into **1 Petri plate**,
Pour about 15 ml of medium
REBECCA™ base (AEB620027 – 6 flasks of 200mL)
or **REBECCA™+ EB (AEB620027 – 6 flasks of 200mL + AEB184135 – supplement for 1.2 L)**
cooled to 44-47°C.

Pour a double layer (5ml) if necessary.

Incubation for 24 ± 2 hours at 37°C

Enumerate characteristic colonies of *E. coli* β-D-glucuronidase +:
blue colonies with or without halo

Retain the plates containing less than 150 characteristic colonies

Refer to EN ISO 7218 standard for calculations and expression of results.

** : Repeat the step with successive dilutions if necessary*

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

Certificate No : AES 10/06 – 01/08

ALTERNATIVE METHODS FOR AGRIBUSINESS

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bioMérieux SA
376 Chemin de l'Orme
69280 Marcy-l'Etoile - France

673 620 399 R.C.S LYON
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

Enumeration of Enterobacteriaceae

REBECCA™ + EB Method

Dilution

X g or **X ml** of sample **+ 9 X ml** of **Buffered Peptone Water** or **Peptone Salt**
or refer to EN ISO 6887 standard for the specific preparations
Carry out successive dilutions if necessary

INOCULATION BY SURFACE METHOD

Spreading of 0.1 ml* on 1 plate of medium

REBECCA™ + EB (AEB520020 – 20 plates Ø 90mm)

Previously dried in an incubator

To increase the accuracy, possibility to spread 1 ml* onto 3 plates Ø 90 mm (see EN ISO 7218 standard).

Incubation for 24 ± 2 hours at 37°C

INOCULATION BY POUR-PLATE METHOD

Inoculate **1 ml*** in **1 Petri plate**,
Pour about 15 ml of medium
REBECCA™+ EB (AEB620027 – 6 flasks of 200mL + AEB184135 – supplement for 1.2 L)
cooled to 44-47°C.
Pour a double layer (5ml) if necessary.

Incubation for 24 ± 2 hours at 37°C

Enumerate characteristic colonies of *Enterobacteriaceae*:
blue colonies with or without halo (*E.coli* β-D-glucuronidase +)
and
pink to red colonies (*Enterobacteriaceae* others than *E.coli* β-D-glucuronidase +).

Retain the plates containing less than 150 characteristic colonies

Refer to EN ISO 7218 standard for calculations and expression of results.

** : Repeat the step with successive dilutions if necessary*

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

Certificate No : AES 10/07 – 01/08

ALTERNATIVE METHODS FOR AGRIBUSINESS

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69280 Marcy-l'Etoile - France

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Fax 33 (0)4 78 87 20 90
www.biomerieux.com