RIDA® Anreicherungsbouillon

REF Z1000

R-Biopharm AG, An der neuen Bergstrasse 17, 64297 Darmstadt, Germany
+49 (0) 61 51 81 02-0 / +49 (0) 61 51 81 02-20 / www.r-biopharm.com

1. Intended use

For *in vitro* diagnostic use. The RIDA® Anreicherungsbouillon is used for the enrichment of bacteria that produce verotoxin 1 and verotoxin 2 (synonyms: Shiga toxin 1 and Shiga toxin 2) and for inducing the production of those toxins in untreated human stool samples from people with symptoms of gastroenteritis.
The RIDA® Anreicherungsbouillon is used for sample preparation and is intended for use with the RIDASCREEN® Verotoxin kit.
The product is intended for professional use.

2. Summary and explanation of the test

The RIDA® Anreicherungsbouillon is offered as an accessory for the RIDASCREEN® Verotoxin test (see Section 1. Intended use).

3. Test principle

The RIDA® Anreicherungsbouillon selectively supports the enrichment of *E. coli* through its proportion of bile salts and suppresses the growth of Gram-positive bacteria. The addition of mitomycin C induces verotoxin production and its release through cell lysis, allowing the verotoxins to be reliably detected in the supernatant of the enrichment culture in the subsequent screening using the RIDASCREEN® Verotoxin test, even when there is little toxin production.

4. Reagents provided

The reagents in the kit are sufficient for 100 determinations.

Table 1: Reagents provided

Kit components	Amount	Description
mTSB-Bouillon	100 assays	Vials with 4 mL enrichment broth each; yellow colored, not reusable

5. Storage instructions

Follow the handling guidelines in Table 2 and store the remaining enrichment broth directly after use according to the information specified. After the expiration date, the quality guarantee is no longer valid.

Microbial contamination of the enrichment broth can render the broth unusable and is to be avoided.

Visible turbidity of the clear, light-yellow enrichment broth is a sign of microbial contamination. Do not use such vials any longer, and properly dispose of them.

Table 2: Storage conditions and information

	Storage temperature	Maximum storage time	Additional notes on storage
unopened vials	2 - 8 °C	Can be used until the printed expiration date	Avoid exposure to direct light. Store unneeded reagents refrigerated.

6. Reagents required but not provided

6.1 Reagents

The following reagents are needed for the evaluation:

Reagents
RIDASCREEN® Verotoxin

6.2 Laboratory equipment

The following equipment is needed for the enrichment of pathogens using the RIDA® Anreicherungsbouillon:

Equipment
Disposable pipettes or disposable inoculation loop/spatula
Micropipette for 100 µL volumes
Cotton wads (optional)
Horizontal shaker or rotary mixer with rack for vials of size 16.5 x 105 mm
37 °C incubator

7. Warnings and precautions for the users

Only qualified laboratory personnel may use this product.
The guidelines for working in medical laboratories (good laboratory practice) must be followed. Adhere strictly to the operating manual when using the RIDA® Anreicherungsbouillon. Do not pipette samples or reagents using your mouth. Avoid contact with broken skin and mucous membranes. Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test. Do not smoke, eat, or drink in areas where samples are handled.

Hazardous materials are indicated according to labeling obligations.
Further details on the Safety Data Sheet (SDS) can be found under the item number at https://clinical.r-biopharm.com/search/.

Users are responsible for the proper disposal of all reagents and materials after use. For disposal, please adhere to the relevant national regulations.

Do not freeze the kit. Do not use a kit that has been frozen.

For users in the European Union: Report all serious adverse events associated with the product to R-Biopharm AG and the relevant national authorities.

8. Collection and storage of samples

Collect stool samples in clean standard containers. Do not collect the stool samples in transport containers that contain transport media with preservatives or fixatives, animal sera, metal ions, oxidizing agents, or detergents because negative effects can result. If rectal smears are used, make sure that the amount of stool material is sufficient for the test (approximately 100 mg).

Follow the sample storage instructions in Table 3. It is recommended not to freeze the stool sample because afterward the verotoxin-producing *E. coli* (VTEC) will not be able to reproduce adequately or at all in the enrichment broth.

Table 3: Sample storage

Stool sample		Enriched stool sample	
20 - 25 °C	2 - 8 °C	2 - 8 °C	≤ 25 °C
≤ 5 days	≤ 5 days	≤ 5 days	≤ 5 days

9. Test procedure

9.1 General information

For successful enrichment, follow the procedure described below and adhere to the specified pipette volumes as precisely as possible. Do not reuse used enrichment broth for further enrichment.

Bring the enrichment broth to room temperature (20 - 25 °C) before use.
Always mix all stool samples thoroughly before use.

9.2 Enrichment of samples

Liquid samples
If the stool sample is liquid, use a disposable pipette to remove about 100 µL and resuspend the sample in the mTSB-Bouillon.

Solid samples
For solid stool samples, use a spatula or disposable inoculation loop to collect 50 - 100 mg and resuspend the sample in the mTSB-Bouillon.

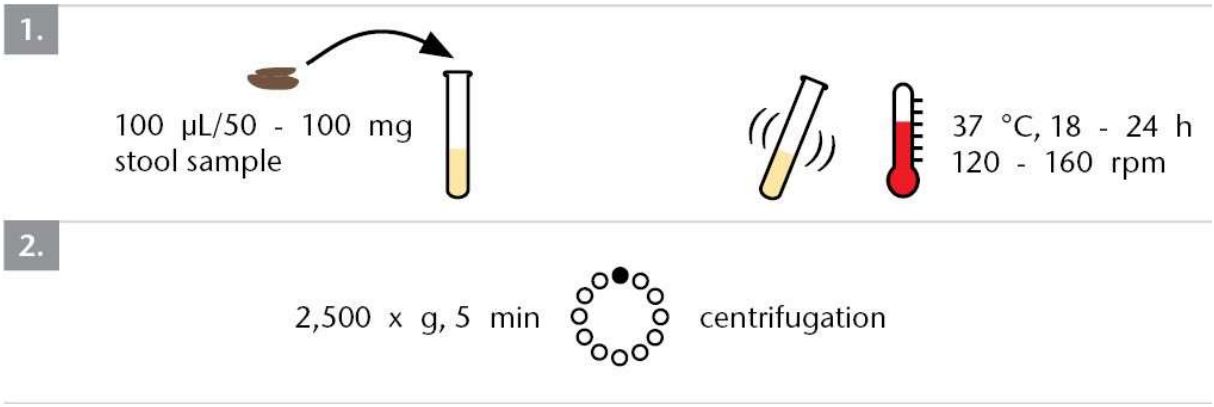
Single colonies from pure culture

Suspend a single colony from pure culture in the mTSB-Bouillon.

Enrichment
Incubate the inoculated mTSB-Bouillon at an angle for 18 - 24 hours at 37 °C under shaking (120 - 160 rpm) and adequate oxygen supply (half turn of the screw cap). Ensure that no liquid escapes.
The horizontal shaker and rotary mixer are equally suitable for shaking.
After no more than 24 hours, centrifuge the enrichment broth at 2500 x g for 5 minutes. Of the supernatant, use 100 µL undiluted in the RIDASCREEN® Verotoxin ELISA.

Important: If a biofilm forms on the enrichment broth, the biofilm should be carefully removed so that it is not transfered to the microtiter plate. This biofilm can cause false-positive results due to its high adherence to the wells of the microtiter plate.

9.3 Short protocol



1. Resuspend the homogenized sample (100 µL for liquid stool sample and 50 - 100 mg for solid stool sample) in 4 mL mTSB-Bouillon and incubate at an angle for 18 - 24 hours at 120 - 160 rpm and 37 °C under adequate oxygen supply.
2. Centrifuge at 2500 x g for 5 minutes and then use 100 µL supernatant undiluted in the RIDASCREEN® Verotoxin ELISA.

10. Limitations of the method

1. The RIDA® Anreicherungsbouillon is intended only for use with the RIDASCREEN® Verotoxin test.
2. This product should be used in compliance with the regulation on good laboratory practice (GLP). When using the RIDA® Anreicherungsbouillon, operators must precisely follow the manufacturer's instructions for the RIDASCREEN® Verotoxin test.

11. Version history

Version number	Section and designation
2016-08-08	Previous version
2021-11-17	General revision 1. Intended use 2. Summary and explanation of the test 3. Test principle 4. Reagents provided 5. Storage instructions 6. Reagents required but not provided 7. Warnings and precautions for the users 8. Collection and storage of samples 9. Test procedure 10. Limitations of the method 11. Version history 12. Explanation of symbols

12. Explanation of symbols

General symbols

	For *in vitro* diagnostic use
	Observe operating manual
	Batch number
	Use before
	Storage temperature
	Item number
	Number of tests
	Date of manufacture
	Manufacturer
	Do not reuse

Test-specific symbols

mTSB Bouillon	Enrichment broth vials