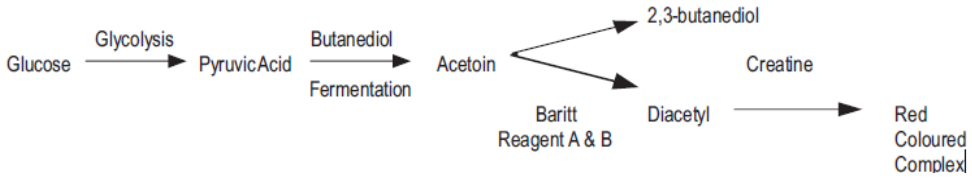
Barritt Reagent A

Intended Use
The reagent is used in Voges-Proskauer test for detection of acetoin production by bacterial culture.

Summary
The fermentation of glucose by bacteria results in end products that vary from species to species depending on metabolic pathways that are available to them under the culture conditions. Many bacteria ferment glucose utilizing the butylene glycol pathway to produce acetyl methyl carbinol (acetoin) or its reduction product 2,3-butanediol. Since 2,3-butanediol cannot be detected directly, the VP test detects the production of acetyl methyl carbinol (acetoin), a natural product formed from pyruvic acid in the course of glucose fermentation. Baritt Reagent A and B are added to the medium along with Creatine which acts as a catalyst. The development of a red colour is a positive reaction for the production of acetoin.

Principle
The VP test is used to identify microbes that ferment glucose and produce neutral, non-acidic end products. After glycolysis (glucose fermentation), these organisms convert pyruvic acid formed into acetyl methyl carbinol (acetoin) or its reduction product 2,3-butanediol. Since 2,3-butanediol cannot be detected directly, this test indirectly determines its presence by testing for acetoin. Acetoin is oxidized to diacetyl in the presence of Baritt Reagent A (α- Naphthol) and Baritt Reagent B (Potassium Hydroxide). Diacetyl, in turn reacts with Creatine, which acts as a catalyst to produce a red coloured complex.



Reagents/contents
The Microxpress® Baritt A and B are reagents set for laboratory use only.
The Microxpress® Baritt A and B reagents comprise of:
1. Baritt Reagent A: 5% α- Naphthol in absolute alcohol.
2. Baritt Reagent B: 40% Potassium Hydroxide.

Storage and Stability
1. Store the reagents at 15°C-25°C away from light.
2. Stability of the reagents is as per the expiry date mentioned on the label.

Procedure
Preparation of Inoculum
1. Isolate the organism to be identified on Nutrient Agar or Brain Heart Infusion Agar.
2. Pick up a single isolated colony and inoculate it in 4-5 mL Brain Heart Infusion Broth.
3. Incubate at 37°C for 6-8 hours until inoculum turbidity is between 0.1- 0.2 at 620 nm. Alternatively, a homogenous suspension made in 2-3 mL sterile saline adjusted to a turbidity of 0.1- 0.2 at 620 nm can also be used as inoculum.

Test Procedure
1. Inoculate an aliquot (0.2 mL) of a suitable medium like MR-VP broth with the above-prepared inoculum (approx. 100 mL) and incubate for 6-8 hours at 35°C-37°C.
2. Observe for growth.
3. Add 1 - 2 drops of Creatine, 2 - 3 drops of Baritt Reagent A and 1 - 2 drops of Baritt Reagent B to it.
4. Shake well after the addition of each reagent to aerate the sample.
5. Observe for the absence or presence of colour change.

Appearance: Amber coloured clear liquid.

Interpretation of results

1. Formation of a red colour complex within 5-10 minutes is indicative of a positive reaction.
2. No change in colour/copper colour is indicative of a negative reaction.

Quality control

Organisms (ATCC)	Observed VP test result
Klebsiella aerogenes (13048)	+
Escherichia coli (25922)	-

Key:

+ =Red colouration
- = No colour change

Precautions/limitations

1. The Baritt A and B reagents are used *in-vitro* diagnostics and for laboratory, professional use only. Not for medicinal use.
2. The Baritt A and B reagents cannot be used directly on clinical specimens. Only pure cultures should be used to obtain optimum results.
3. After exposure to the reagents for over one hour, a negative culture may show a copper-like colour due to the action of potassium hydroxide on the α - Naphthol. This is not a positive reaction.
4. Reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur.
5. At times, the organism may give contradictory results because of mutation or media used for isolation, cultivation and maintenance. Results are prominent when fresh and enriched culture is used.
6. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
7. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of gram reaction, morphology, and other biochemical and serological tests.
8. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

1. Practical Medical Microbiology, Mackie & McCartney, 13th edition 1989, Edited by J. G. Collee, J. P. Duguid.
2. Clinical Diagnosis & Management by Laboratory Methods, Todd, Sanford and Davidsohn, 17th edition 1998, Edited by John Bernard Henry.
3. Two quick methods for Voges-Proskauer test by A. L. Barry and K. L. Feeney, Applied Microbiology, Sept. 1967, p.: 1138-1141.
4. Effect Of Acetate Upon The Formation Of Acetoin In Klebsiella And Enterobacter And Its Possible Practical Application In A Rapid Voges-Proskauer Test. Applied Microbiology, Mar. 1973, Vol. 25, No. 3, P: 511-512.
5. Coblentz, L.H 1943, Rapid detection of the production of acetyl-methyl-carbinol, Am. J. Pub. Health 33:815-817.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Pack Size
204020570100	Barritt Reagent A	100 mL