# VOGES-PROSKAUER REAGENTS

## (α-NAPHTHOL, 40% POTASSIUM HYDROXIDE & CREATINE)

- For in vitro use only -

Catalogue No.
RA45, RP89, & RC90

Our Voges-Proskauer (VP) Reagents when used with MR-VP Broth can differentiate organisms based on their ability to produce the end product, acetylmethylcarbinol (acetoin), from glucose fermentation. This characteristic can be used to aid in the differentiation between genera

1. *Klebsiella pneumoniae* subsp. *pneumoniae* (+) and *Enterobacter* (usually +) from *E. coli* (-)
2. *Staphylococcus* (usually +) from *Micrococcus* (-)

And aid in species differentiation

1. *Klebsiella pneumoniae* subsp. *pneumoniae* (+), *K. oxyoca* (+), *K. planticola* (+), *K. terrigena* (+) from *K. pneumoniae* subsp. oz*aenae* (-) and *K. pneumoniae* subsp. *rhinoscleromatis* (-)
2. *Aeromonas hydrophilia* (+), *A. salmonicida* subsp. *masoucida* (+), *A. veronii* (+), and *A. sobria* (usually +) from other commonly isolated *Aeromonas* spp. (-)
3. Viridans streptococci: *S. mutans* (+), *S. milleri* group (variable), *S. bovis* (+), and *S. salivarius* (usually +) from *S. sanguis* (-) and *S. mitis* (-)

The Voges-Proskauer test is usually performed alongside the methyl red test since both tests are performed on cultures grown in MR-VP broth. Both tests are based on the detection of end products from the metabolism of glucose. Bacteria can metabolize glucose to the key intermediate, pyruvic acid. Numerous pathways can further metabolize pyruvic acid, and acetoin is produced as an end product from one of these pathways. The VP test is based on the detection of acetoin, and its detection is achieved by the addition of various reagents that lead to the formation of colored end products.

The VP test is credited to Voges and Proskauer since they were the first bacteriologists to observe a red color change on culture media after treatment with potassium hydroxide. Barritt was the first individual to use both potassium hydroxide and α-naphthol for the VP test; an alternate, rapid VP test was reported by Barry and Feeney using an additional reagent, creatine.

The first reagent added, α-naphthol, catalyzes the conversion of acetoin to diacetyl in the presence of oxygen. Diacetyl can then react with guanidine-containing compounds such as arginine in the presence of α-naphthol to form a pinkish-red end product. The resultant red color change is indicative of a positive VP test.

The second reagent, potassium hydroxide, absorbs carbon dioxide present in the medium and acts as an oxidizing agent thereby hastening the critical reaction that converts acetoin to diacetyl.

For the rapid VP test, creatine is added as an additional source of guanidine nucleus. Once added, creatine can react with diacetyl to promote color development. Also, the overall structure of creatine, which possesses an additional free $NH_2$ group, intensifies color development.

**Formulation per 100 mL**

RA45 α-Naphthol Reagent
α-Naphthol ..... 5.0 g

RP89 Potassium Hydroxide (40%)
Potassium Hydroxide ..... 40.0 g
Sterile De-ionized Water ..... 100.0 mL

RC90 Creatine Reagent (0.5%)
Creatine..... 0.5 g
Sterile De-ionized Water ..... 100.0 mL

**Recommended Procedure**

Conventional Procedure:

1. Inoculate a tube of MR-VP broth (Dalynn TM85) with the organism of interest from a overnight culture grown on KIA, TSI Agar, MacConkey Agar, or Blood Agar.

2. Incubate for 24 hours at 35°C. (The VP test can also be performed at 48 hours)
3. Remove 1.0-mL of the incubated broth to a separate tube for VP testing. (The remainder of the broth should be re-incubated for an additional 1-3 days for the methyl red test)
4. Allow reagents to warm to room temperature prior to use.
5. Add 0.6 mL (9 drops) of α-Naphthol Reagent to the allotted portion of MR-VP broth and gently mix.
6. Add 0.2 mL (3 drops) of 40% Potassium Hydroxide.
7. Shake the tube gently for 30 seconds. The broth must be exposed to oxygen for a color reaction to occur.
8. Allow tube to stand for 15 minutes before interpreting.

Rapid VP Procedure:

1. From a stock solution of MR-VP Broth, aseptically pipette 0.2-mL aliquots into sterile test tubes (10x75 mm) just prior to use.
2. Take a loopful of growth from an overnight culture grown on KIA, TSI Agar, MacConkey Agar, Blood Agar, or Chocolate Agar and inoculate the broth.
3. Incubate tube at 35°C (water bath) for 4 hours.
4. Add 2 drops of Creatine Reagent and gently mix.
5. Add 3 drops of α-Naphthol Reagent and gently mix.
6. Add 3 drops of 40% Potassium Hydroxide and gently mix for 10 seconds.
7. Allow tube to stand for 15 minutes before interpreting the color.

**Interpretation of Results**

VP Positive: Pink or red color at the surface of the medium
(acetoin present)

VP Negative: Yellow or copper color at the surface of the medium
(acetoin absent)

- *Ensure that the reagents are added in the correct sequence; if added first, KOH can react with the peptone in the medium to yield a salmon-pink color that could be misinterpreted as a positive result*
- *Aeration of the broth by shaking greatly enhances the oxidation of acetoin to diacetyl and color development as well*
- *VP tests performed on cultures after 72 hours of incubation may yield weakly-positive or false-negative results due to acid interference*
- *Some bacteria, such as Hafnia alvei, are VP variable at 35°C, but VP positive at 25-30°C*
- *Most members of the family Enterobacteriaceae give opposite methyl red and VP reactions, but not in all instances. Hafnia alvei and Proteus mirabilis may give both a positive methyl red reaction and a positive VP reaction*
- *Do not read test results more than one hour after adding the reagents*

**Quality Control**

After checking the medium for correct pH, color, depth, and sterility, the following organisms are used to determine the performance of the completed medium.

| Organism | MR | VP |
|---|---|---|
| *Enterobacter aerogenes* ATCC 12453 | – (yellow) | + (red) |
| *Escherichia coli* ATCC 25922 | + (red) | – (no change) |

## Storage and Shelf Life

Our Potassium Hydroxide (40%) Reagent should be stored at 4 to 8°C. At this temperature it has a shelf life of 26 weeks from the date of manufacture.

Our α-Naphthol Reagent can be stored at room temperature for an indefinite period of time in its dry powdered form. Each bottle contains 0.5-g of α-naphthol and 10-mL of ethanol must be added to solubilize the α-naphthol before using it. From this point on, the alcoholic solution has a shelf life of 16 weeks.

Our Creatine Reagent should be stored at 4 to 8°C. At this temperature it has a shelf life of 26 weeks from the date of manufacture.

## Ordering Information

| Cat# | Description | Format |
|---|---|---|
| TM85-02 | MR-VP Broth 2-mL [13x100-mm Screw Cap Tube] | 10/pkg |
| TM85-06 | MR-VP Broth 6-mL [16x100-mm Screw Cap Tube] | 10/pkg |
| RM65-25 | Methyl Red Reagent 25-mL | Each |
| RA45-10 | Alpha Naphthol Reagent 10-mL | Each |
| RP89-25 | Potassium Hydroxide [40%] 25-mL | Each |
| RC90-25 | Creatine Reagent [0.5%] 25-mL | Each |

## References


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5. Barry AL, Feeney KL. Two quick methods for Voges-Proskauer test. Appl Micro 1967; 15:1138-41.
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