CATALASE REAGENT

- For in vitro use only - Catalogue No. RC35

Our Catalase Reagent is used to detect the presence of the enzymes, catalase and peroxidase, produced by some bacteria.

In the respiratory chain of all bacteria, reduced flavoproteins and iron-sulfur proteins unite with oxygen and oxidases to form two compounds, hydrogen peroxide and the superoxide radical. These compounds, if allowed to accumulate, are toxic to bacteria and results in their death. Bacterial survival is accomplished by the production of specific enzymes that allow bacteria to neutralize the toxic compound.

Hydrogen peroxide is decomposed by the action of two enzymes: catalase and either a peroxidase, NADH, NADPH, cytochrome c, or glutathione. To observe the action of these enzymes, catalase reagent, a dilute solution of hydrogen peroxide, is added to a pure bacterial culture. Any immediate bubbling is indicative of a positive result since oxygen is a byproduct of hydrogen peroxide decomposition.

Our catalase reagent can be used:

To differentiate between genera:

1. *Streptococcus* (–) from *Micrococcus* (+) and *Staphylococcus* (V+)
2. *Bacillus* (+) from *Clostridium* (V–)
3. *Listeria monocytogenes* (+), *Kurthia* (+), *Corynebacterium* (+) from other microorganisms that may be similar morphologically: *Erysipelothrix* (–), *Lactobacillus* (V-), *Enterococcus* (–), and group B *Streptococcus* (–)
4. *Kingella denitrificans* (–), *Neisseria elongata* subsp. *elongate* (–), and *Neisseria elongata* subsp. *nitroreducens* (–) from other *Neisseria* (+) and *Moraxella catarrhalis* (+)
5. *Xenorhabdus* spp. (–) from other *Enterobacteriaceae*
6. *Clostridium histolyticum*, *C. carnis*, *C. tertium* (all -) from other aerobic bacilli (+)

To aid in species differentiation:

1. *Aerococcus urinae* (+) from *Aerococcus viridans* (–)
2. *Pediococcus acidilactici* (+) and P. *pentosaceus* (+) from other *Pediococcus* (–)
3. *Staphylococcus aureus* subsp. a*naerobius* and *S. saccharolytica* (–) from other *Staphylococcus* spp. (+)
4. *Campylobacter fetus*, *C. hyointestinalis*, *C. jejuni*, and *C. coli* (all +) from *C. spurorum*, *C. concisus*, and *C. mucosalis* (all –)
5. *Neisseria elongata* subsp. *elongata*, *N. elongata* subsp. *nitroreducens*, and *N. mucosa* (all –) from other *Neisseria* spp. (+)
6. *Moraxella bovis* (variable) and *Kingella* spp. (–) from other *Moraxella* spp. (+)
7. *Prevotella oulorum* (+) from other *Prevotella* spp. (–)

Formulation per 100 mL

Hydrogen peroxide (3%) ..... 100.0 mL

Recommended Procedure

A. Slide Method

1. With a sterile inoculating loop or wooden applicator stick, pick the center a colony derived from an overnight culture plate and place it on a clean, glass slide.
2. Place a drop of catalase reagent onto the smear.
3. Observe for immediate bubbling. It may be necessary to use a hand lens to detect weakly positive reactions.

B. Tube Method

1. Add 1.0-mL of Catalase Reagent directly to an overnight, heavily inoculated pure agar slant culture. (Do not use a blood agar)
2. Observe for immediate bubbling.

- *For the slide method, do not reverse the order of the procedure as platinum needles may produce false positive results. Nichrome wire does not cause bubbling*
- *Reactions are immediate although weak reactions may be difficult to observe without a hand lens*
- *The concentration of hydrogen peroxide in our Catalase Reagent is 3%; some catalase test procedures require a 30% solution of hydrogen peroxide. Do not confuse the two concentrations*
- *Colonies obtained from blood agar plates should be picked and transferred very carefully. Any accidental transfer of the medium to the slide will result in false positive results since erythrocytes (RBCs) contain catalase/peroxidase. To avoid this problem chocolate agar can be used*
- *Growth for catalase testing must be from an 18 to 24-hour culture since older colonies may lose their catalase activity and yield false-negative results*
- *Be aware that catalase-negative isolates may exist for many species*
- *When anaerobes are tested, cultures should be exposed to air for 30 minutes prior to the addition of catalase reagent*
- *Catalase is inactivated by sunlight*

Interpretation of Results

Positive: Immediate bubbling
(Oxygen is released)

Negative: No bubbling

Quality Control

Organism	**Expected Results**	
Staphylococcus aureus ATCC 25923	+ve	Bubbling
Streptococcus pyogenes ATCC 19615	-ve	No bubbling

Storage and Shelf Life

Our Catalase Reagent should be stored at 4 to 8°C and protected from light. Under these conditions, the reagent has a shelf life of 26 weeks from the date of manufacture.

References

1. Mitchell RL, Anderson IC. Catalase photoactivation. Science 1965; 150:74.
2. Funada H, Hattori K-I, Kosakai N. Catalase-negative *Escherichia coli* isolated from blood. J Clin Micro 1978; 7:474-8.
3. Chester B, Moskowitz LB. Rapid catalase supplemental test for identification of members of the family *Enterobacteriaceae*. J Clin Micro 1987; 25:439-41.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol I. Washington, DC: ASM, 1992.
5. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington DC: ASM Press, 1999.
6. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

Original: April 2002
Revised / Revisited: January 2014