# **GRIESS-ILOSVAY's Nitrite Reagent**

Reagent solution for detecting microbial nitrite in order to identify nitrate-reducing microorganisms.

#### Mode of Action

GRIESS-ILOSVAY's reagent reacts with nitrate to form a red diazo dye. If high concentration of nitrate are present, the colour of the dye may change to yellow.

#### **Typical Composition**

Sulfanilic acid; 1-naphthylamine; acetic acid.

## **Experimental Procedure and Evaluation**

The pure culture of the microorganism to be tested is inoculated into Nitrate Broth or into Standard II Nutrient Broth to which 1.5g potassium nitrate (Merck, Cat. No. 1.05063.) have been added. Incubate for 12-24hours at the optimal temperature.

After incubation, add several drops of the nitrite reagent to the culture. If nitrite is present an intense red colour appears within one minute, the intensity of the colour is proportional to the quantity of nitrite formed. In case of microorganisms which produce large amounts of nitrite, the initial red coloration changes to yellow.

A negative reaction signifies that nitrate has not undergone any reaction or that is has been reduced to nitrogen of ammonia. The "zinc dust test" must be then performed to verify which of these reactions have been taken place.

Zinc dust test: If the result of GRIESS-ILOSVAY test is negative, add a quantity of zinc dust (Merck, Cat. No. 1.08774.) about the size of a pepper corn for every 5 ml of culture medium and allow to settle without shaking. If nitrate is present, the medium surrounding the zinc dust becomes pink. The nitrate is reduced to nitrite which can then react with the GRIESS-ILOSVAY's reagent. A positive zinc dust test signifies "no nitrate reduction"; a negative zinc dust test signifies "nitrate has been reduced".

Nitrate may not be reduced, if the zinc particles are coated with a layer of patina. The zinc dust should therefore be as fresh as possible.

Reaction	Nitrate Reduction
GRIESS-ILSOVAY: positive negative → Zinc dust test: negative:	positive positive
positive:	negative

### **Ordering Information**

Product	Merck Cat. No.	Pack contents
GRIESS-ILOSVAY's Nitritre Reagent	1.09023.0500	500 ml