

ENTEROCOCCUS MICRO TESTER PRO WATER TEST KIT

STIB007

Enterococcus Presumptive Identification Test for Fresh Water, Marine water and laboratory cultures.

BRINGING THE LABORATORY TO THE FIELD

Simpltek brings the testing laboratory where it is needed most—the field. Our tests are easy to use, fast and reliable, have an extended shelf life and present a unique, cost-effective way to perform microbiological analysis in the field or the laboratory.

Our tests allow you to identify microorganisms that traditionally could only be cultured or identified using expensive equipment in a laboratory setting for a fraction of what a laboratory charges. When using Simpltek tests, you can make the decisions in the field, where they count.

Read all directions entirely before running this test.

SUMMARY AND EXPLANATION

Simpltek Enterococcus Test is an enterococcus selective broth has the same formula as Enterococcosel™ Agar with the agar omitted in a concentrated form. Water samples suspected of containing Enterococcus faecalis are inoculated into the ampoule, rehydrating the media and then incubated at 95 °F for 8-24 hours. The combination of esculin and a rather low concentration of bile in the presence of azide permit the selection of enterococci and differentiation by esculin hydrolysis (blackening of the medium). Enterococci hydrolyze esculin to produce esculetin, which reacts with the ferric ammonium citrate to form a dark brown or black complex. Oxgall inhibits gram-positive bacteria other than enterococci. Sodium azide is inhibitory for gram-negative Microorganisms.

PRESENCE/ABSENCE METHOD

If using the supplied sample cup, triple rinse the cup with the water sample prior to use.

- Gather the sample in the supplied sample cup filling to the 25 mL line or preferably in a single use sterile sample container.
- (Optional) If samples contain chlorine, add 5 drops of the De-chlorination solution for every 25 mL of sample. Swirl to mix and let sit 2 minutes.
- Remove ampoule from box and carefully remove and save the provided safety cap. Inspect the ampoule tip for breakage. If broken discard properly and get a new ampoule.
- With an unbroken ampoule, place the tip (without safety cap) in the sample container with the tip against the sample container wall holding the ampoule at a 45° angle. Gently push the tip against the sample container wall with a slight twisting motion. The ampoule tip will break and the sample will automatically be drawn into the ampoule. Make sure to keep the ampoule tip in the sample until it has finished filling.
- Remove the ampoule from the sample. Incubate the ampoule at 95 °F. Check hourly for a black precipitate. When the black precipitate develops, record the time in hours from start of the test to the appearance of the black precipitate and check the chart below to estimate bacterial levels.
- Sterilize sample container with 10% bleach before next test. For example, to a 20 mL bacterial sample, add 2 mL or ~40 drops bleach solution. Mix and wait for 10 minutes. Rinse 3 times with sterile milli-Q water to remove residual bleach.
- If the test result is positive, estimate the bacteria population by referring to the chart below.

Approximate bacteria population:

Elapsed Time (hrs)	Approximate bacteria population (CFU/mL)	Contamination Severity
6	$>10^8$	Very High
8	$10^6 - 10^7$	High
10	$10^5 - 10^6$	High
12	$10^4 - 10^5$	Moderate
16	$10^3 - 10^4$	Moderate
18	$10^2 - 10^3$	Moderate
24	1 – 10	Low
30	< 1	Low

EXPECTED RESULTS

Positive growth is indicated by the formation of black precipitates.

LIMITATIONS OF PROCEDURE

For final identification in the laboratory using The Simpltek Enterococcus Test, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.

STORAGE

Upon receipt, store tubes in the dark at 35-70 °F. Avoid freezing and overheating. Ampouled media stored as indicated may be inoculated up to the expiration date. Minimize exposure to light.

PRODUCT DETERIORATION

Do not use ampoules if they show evidence of microbial contamination, discoloration, or other signs of deterioration.

EXPIRATION DATE

The product is stable if stored properly for 4+ years from manufacture. The expiration date applies to media stored at or below 70 °F without direct exposure to light.

LABORATORY QUALITY CONTROL

Incubate the listed bacterial strains for about 16 hours, until reach 2.0 absorbance @600nm ($\sim 10^9$ CFU/mL).

Perform CFU counting to determine the cell density of bacterial cultures.

Dilute the mixed bacterial culture with sterile phosphate buffered saline (PBS) to the final CFU/mL of 10^8 , 10^7 , 10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10, and 1. Use sterile PBS buffer as negative control.

Inoculate ampoules with these bacterial cultures and incubate in an aerobic environment at 95 °F. Perform biological triplicates for each cell density and negative control.

Examine the color development of all inoculated ampoules and record the time of the appearance of pink color up to 24 hours post inoculation.

QUALITY CONTROL ORGANISMS

Enterococcus faecalis ATCC 29212

Growth-Blackening of media.

Enterococcus faecalis ATCC 10741

Growth-Blackening of media.

Streptococcus pyrogenes ATCC 19615

Inhibition (partial), No black reaction.

Escherichia coli ATCC 25922

Inhibition (partial), No black reaction.

WARNING AND PRECAUTIONS

- For *in vitro* Diagnostic Use.
- For laboratory and field use by trained professionals.
- The Simplatek Enterococcus Test uses a glass ampoule with a sharp tip when activated. USE EXTREMECAUTION when breaking the tip. Always carefully apply the provided safety cap.
- Dispose of broken unused ampoules in a broken glass receptacle.
- Dispose of used ampoules in an appropriate sharps container or sealed puncture resistant receptacle then offer for biohazard processing according to local, state and Federal regulations.
- Keep away from children.
- Not for use as a diagnostic tool on humans or animals.
- Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures before, during and after use.
- Prepared ampoules, specimen containers and other contaminated materials must be sterilized by autoclaving or disinfectant solution before discarding.

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