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Acid Phosphatase Reagent

Reagent used for the confirmation of *Clostridium perfringens* in water samples

Culture media | Clostridium perfringens | Water analysis | DIN EN ISO 14189



Acid Phosphatase Reagent

Reagent used for the confirmation of *Clostridium perfringens* acc. to DIN EN ISO 14189

Acid phosphatase activity is a characteristic biochemical feature of Clostridium perfringens.
The enzyme hydrolyzes specific substrates in the culture medium, resulting in a characteristic color reaction.
The detection of clostridia in water samples is used for the hygienic assessment of drinking water, recreational water, and surface water.

In particular, *Clostridium perfringens* is regarded as an important indicator of fecal contamination and historical pollution events, as its spores exhibit high resistance to environmental stressors and disinfection measures. The combination of selective cultivation and enzymatic detection enhances the reliability of identification.

- **No in-house reagent preparation required**
  thanks to confirmation testing with the Acid Phosphatase Reagent
- **Clear confirmation of *Clostridium perfringens***
  through the development of dark red to reddish-brown colonies
- **The Acid Phosphatase Reagent facilitates microbiological analyses in accordance with applicable standards**



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Detection workflow for *Clostridium perfringens*

Sample membrane filtration

Transfer of the filter onto TSC Selective Culture Medium TN1241 TN1333

44 ± 1 °C, 21 ± 3 h, anaerobic

Count black or grey to yellow colonies as presumptive *Clostridium perfringens* (including spores).

Two methods are available for the confirmation of *Clostridium perfringens*:

ACID PHOSPHATASE REAGENT

Subcultivation on Blood agar, Columbia agar, or similar culture media TN1118 TN1191 TN1519

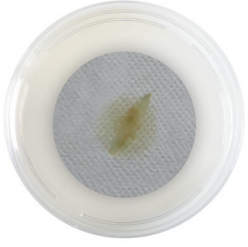
36 ± 2 °C, 21 ± 3 h, anaerobic

BUFFER ADDITION	**Pipette 0.5 ml of buffer into the vial with Dye Reagent**	1 min
VORTEXING	**Vortex until a homogeneous mixture is achieved**	1 min
COOLING	**Incubate the vial for 1 hour at 2-8 °C, protected from light**	60 min
CENTRIFUGE	**Centrifuge at 6,000 rpm for 10 min**	10 min
TRANSFER THE SUPERNATANT	**Transfer 750 µl supernatant into a new vial**	2 min
ADDITION OF ACID PHOSPHATASE REAGENT	**Place 2–3 drops of reagent on filter paper (e.g., in a Petri dish) and rub in a suspected colony**	5 min
REACTION ON FILTER PAPER	**After 3-4 min, the *Clostridium perfringens* colony develops a dark red to reddish-brown coloration**	3-4 min

Positive Result



Negative Result



alternatively

m-CP AGAR

Subcultivation on m-CP Agar TN1288 TN1330

44 ± 1 °C, 21 ± 3 h, anaerobic

Expose to ammonia (ammonium hydroxide) vapors for 20 -30 seconds

The development of a pink to red coloration from initially dark yellow colonies indicates confirmed acid phosphatase detection.

Detection of *Clostridium perfringens*

Clostridium perfringens is an anaerobic, spore-forming bacterium and is considered an important indicator of fecal contamination as well as the effectiveness of water treatment processes.

Cultivation on TSC Selective Culture Medium

Tryptose-Sulfite-Cycloserine (TSC) Agar is used for the selective isolation and enumeration of *Clostridium perfringens*. The bacterium reduces sulfite to sulfide, which, in combination with iron ions, leads to the formation of characteristic black colonies.

Confirmation on m-CP Agar

m-CP Agar (Membrane Clostridium Perfringens Agar) enables selective differentiation. After anaerobic incubation, typical colonies develop a yellow coloration. Subsequent exposure to ammonia vapor results in a characteristic pink to red color change in confirmed *Clostridium perfringens* colonies.

Confirmation with Acid Phosphatase Reagent

Subcultivation is first performed on Blood Agar, Columbia Agar, or similar culture media. Following the reagent-based procedure, confirmed *C. perfringens* colonies turn dark red to reddish-brown within 3-4 min:

Test strain	Reaction	Color Reaction
Clostridium perfringens	+	
Clostridium bifermentans	-	
Clostridium sporogenes	-	
Clostridium sporogenes	-	
Clostridium sordellii	-	

Order Information

Acid Phosphatase Reagent	TN1519	1 x 10 ml Sodium Acetat Buffer 6 x 0.09 g Dye Reagent
Blood Agar (Base)	TN1106	500 g
Columbia Agar (Base)	TN1118	500 g
Columbia Agar acc. to harm. EP/USP/JP	TN1191	500 g
m-CP Agar (Base)	TN1288	500 g
m-CP Selective Supplement	TN1330	4 x 3 btl.
TSC Agar (Base)	TN1241	500 g
TSC Selective Supplement	TN1333	12 x 1 btl.

Materials and equipment not supplied
Filter paper, empty vials or Petri dishes, pipette and pipette tips, spatula, vortex mixer, and centrifuge

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