Technical Info

Well advised with Roth.

Clouding of Microbiological Media

In few rare cases, clouding of microbiological broths happens naturally, resulting from certain ingredients which do not solubilise completely during preparation (for instance cetrimide).
In most cases, clouding is the result of small residues of calcium, which reacts with added phosphates, forming insoluble salts.

1.) Precipitation of Chalk w/o presence of Phosphates

Calcium [water] → Calcium hydrogen carbonate $Ca(HCO_3)_2$ - water soluble (16.6 g/l)

CO_2 [airborn] →

Heating ↓

Calcium carbonate $CaCO_3$

- Practically insoluble in water (0.014 g/l)
- Solubility product (25 °C) 0.87×10^{-8}
- Forms white to light brown crusts

2.) Formation of Insoluble Calcium Phosphates under Presence of Phosphate

Calcium [water] → Calcium hydrogen carbonate $Ca(HCO_3)_2$ - water soluble (16.6 g/l)

CO_2 [airborn] →

Hydrogen phosphates →

Heating ↓

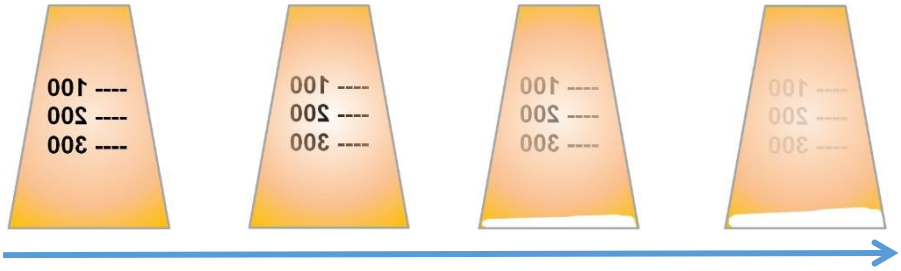
Calcium phosphate $Ca_3(PO_4)_2$

- Practically insoluble in water (0,02 g/l)
- Solubility product (25 °C) 2.07×10^{-33}
- Forms light, white, floating flakes, precipitates only in case of large amounts

Example: Terrific-Broth

Casein, enzymatically digested	12 g/l
Yeast extract	24 g/l
K_2HPO_4	12.5 g/l
KH_2PO_4	2.3 g/l

In small amounts, calcium phosphates are distributed in the broth, only appearing as clouding of the – normally clear – broth. Assayed from the back, the labelling of those bottles or Erlenmeyer flasks appears diffuse and not distinct. Only when they reach a certain critical amount, the phosphates form a visible precipitate, or appear as white clouds.



Increasing concentrations of added phosphates
and/or calcium content in the water

Typically Found in the Following Media (Boths with High Phosphate Content)

TB, Peptone Water, Fraser Half Broth, Fraser Broth, Mossel Enrichment Broth

Rarely Found in the Following Media (Broths with low Phosphate Content)

CASO Bouillon, Coli Fluoro Broth, Brain Heart Infusion Broth, Laurylsulphate Broth, MRS Broth

Please Note

With extracts (particularly yeast or meat extract) phosphates are also added to the broth.

Due to batch variability of those natural products, some batches may form more severe clouding than others.

In Order to Prevent this Effect We Recommend the Following Procedure

A) In the vast majority of cases, phosphate reduction of the medium composition due to precipitation of phosphates doesn't reduce the quality of the broth or bacterial growth rates. In case the clouding doesn't hinder the downstream processes (OD measurement or such), the broth may be used just as it is.

B) In case the precipitates shall be removed, we recommend filtration through filter membranes with large pores (several µm). Please make sure, filtration units and broth are handled sterile.

C) Filtration prior to autoclaving is not sufficient, since due to the heating during steam sterilisation phosphate salts form anew.

D) Try to reduce phosphate concentration of the broth. Instead of Terrific Broth, one can use the phosphate reduced version *Terrific Broth, modified* (Art. No. HP61).

E) Check your water desalting or distillation unit. Analyse the calcium content (water hardness) of your water (see Roth water test sticks).

Solubility Products of Some Phosphates and Other Salts

Salt	Formula	Temperature	Solubility Product ($Mol^2/Litre^2$)
Aluminium hydroxyde (anhydrous)	$Al(OH)_3$	20 °C	$1,9 \times 10^{-33}$
Cadmium phosphate	$Cd_3(PO_4)_2$	25 °C	$2,53 \times 10^{-33}$
Calcium carbonate	$CaCO_3$	25 °C	$0,87 \times 10^{-8}$
Calcium phosphate	$Ca_3(PO_4)_2$	25 °C	$2,07 \times 10^{-33}$
Calcium sulfate	$CaSO_4$	25 °C	$4,93 \times 10^{-5}$
Cobalt phosphate	$Co_3(PO_4)_2$	25 °C	$2,05 \times 10^{-35}$
Iron (II) phosphate	$Fe_3(PO_4)_2 \times 8\ H_2O$	25 °C	$1,70 \times 10^{-29}$
Potassium chloride	KCl	20 °C	21,25
Potassium perchlorate	$KClO_4$	25 °C	$1,05 \times 10^{-2}$
Copper (II) phosphate	$Cu_3(PO_4)_2$	25 °C	$1,4 \times 10^{-37}$
Copper (II) sulphide	CuS	25 °C	8×10^{-37}
Lithium carbonate	$LiCO_3$	25 °C	$1,7 \times 10^{-3}$
Lithium fluoride	LiF	25 °C	$1,84 \times 10^{-3}$
Lithium phosphate	Li_3PO_4	25 °C	$2,37 \times 10^{-4}$
Mercury (II) sulphide	HgS	25 °C	2×10^{-53}

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Copper (II) sulphide	CuS	25 °C	**8×10^{-37}**
Mercury (II) sulphide	HgS	25 °C	**2×10^{-53}**

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