

## 421: CHROMATIUM SALEXIGENS MEDIUM

This recipe contains strain-specific modifications for *Halorhodospira neutriphila* DSM 15116 \*

Final volume: 1000 ml

<b>Main sol. 28</b>	1000.00	ml
NaCl	10.00	%
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.30	%
Na acetate	0.05	%
Sodium Thiosulfate	0.05	%

Supplement medium 28 with NaCl (10%), MgCl<sub>2</sub> x 6 H<sub>2</sub>O (0.3%) Na-acetate (0.05%) and sodium thiosulfate (0.05%). Incubate at 500 to 1000 Lux light intensity.

\* plus 0,1% MgCl<sub>2</sub>x6H<sub>2</sub>O plus 0,15% Na-acetate, Na-thiosulfate, Yeastextrakt plus 5ml 0,01% Vitamine B<sub>12</sub>/l

### Main sol. 28 (from medium 28)

<b>Solution A</b>	460.00	ml
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1. Aliquot Solution A into 100 mL screw-cap bottles, filled with 46 mL each. Bubble with N<sub>2</sub>/CO<sub>2</sub> and autoclave at 121°C for 15 min (as described below).

2. Prepare the following solutions (resazurin, bicarbonate and Pfennig's heterotrophic salts) and sterilize as given below.

<b>Resazurin solution</b>	450.00	ml
<b>Bicarbonate solution</b>	50.00	ml
<b>Pfennig's heterotrophic salts solution</b>	26.00	ml

3. Add bicarbonate solution and Pfennig's heterotrophic salts to the resazurin (complete volumina, i.e. 50 mL bicarbonate solution and 26 mL Pfennig's heterotrophic salts solution). Bubble with CO<sub>2</sub> in an ice bath under sterile conditions.

4. Fill 50 ml of this mixture to each bottle of solution A (46 mL + 50 mL).

5. Before use, add 4 ml sulfide solution (1.5%) and 0.1 ml Vitamin B<sub>12</sub> solution to each 100 mL bottle.

<b>Sulfide solution, 1.5%</b>	40.00	ml/l
<b>Vitamin B<sub>12</sub> solution</b>	1.00	ml/l

6. Adjust the pH with filter-sterilised 1M Na<sub>2</sub>CO<sub>3</sub> to 7.1-7.3.

7. If needed, aliquot into sterile, N<sub>2</sub> gassed screw-cap tubes under N<sub>2</sub> gas.

8. During the first 24 h, the iron of the medium precipitates in the form of black flocks. No other sediment should arise in the otherwise clear medium.

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9. Feed the actively growing culture periodically with neutralized 3% solution of sodium sulfide (use 1 -3 mL/100 mL depending on strain and cultivation stage) to replenish sulfide and with other supplement solutions (see Ref. 3365).

<b>Neutralized sulfide solution 3% (w/v)</b>	10.00	ml
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### **Neutralized sulfide solution 3% (w/v)** (from medium 28)

Na <sub>2</sub> S x 9 H <sub>2</sub> O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H<sub>2</sub>SO<sub>4</sub> drop-wise with a syringe without opening the bottle.