

### ATCC Medium: 2777 *Brockia liithotrophica* Medium

Add approximately 0.06 g powdered sulfur per test tube. Autoclave at 100°C for 30 minutes on 3 consecutive days to sterilize. (Sulfur powder cannot withstand the high heat of the autoclave, so must be sterilized in this manner).

On the 3<sup>rd</sup> day, prepare the medium below:

KCl.....	0.33 g
NH <sub>4</sub> Cl.....	0.33 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.33 g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.33 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.33 g
Starch.....	5.0 g
Yeast Extract.....	0.20 g
Resazurin.....	1 mg
Trace elements (ATCC MD-TMS).....	1 ml
DI Water.....	900 ml

Autoclave at 121°C. After cooling the media, add the following filter sterilized solution:

NaHCO <sub>3</sub> .....	0.5 g
Na <sub>2</sub> S·9H <sub>2</sub> O.....	0.5 g
Vitamin solution (ATCC MD-VS).....	1 ml
DI Water.....	99 ml

Adjust complete medium to pH 6.5 with H<sub>2</sub>SO<sub>4</sub>. Prepare under a head space of 80% H<sub>2</sub> – 20% CO<sub>2</sub>.