Table 1. SP4 growth medium composition (per 1L)

SP4 growth medium, pH 7.0		
Component	Amount	Company/Details
Basal SP4, autoclaved	300 ml	-
20% yeastolate	10 ml	Gibco/Bacto TC yeastolate
15% yeast extract	35 ml	Carl Roth
7.5% $NaHCO_3$	14.6 ml	Honeywell
20% glucose	25 ml	Carl Roth
400000 U/ml penicillin G, Na-salt	2.5 ml	Carl Roth/Cellpure > 1550 U/mg, Penicillin G Na-salt
25 mg/ml L-glutamine	5 ml	Carl Roth/> 99% purity, Cellpure
Fetal Bovine Serum	170 ml (17%)	Biowest/Heat inactivated, sterile filtered
CMRL (if liquid)	400 ml	PAN Biotech/ Liquid CMRL-1066, no glutamine, with 2.2 g/L $NaHCO_3$
CMRL (if powder, no $NaHCO_3$)	3.92 g + 0.88 g powder $NaHCO_3$	Himedia AT226A-20L

To prepare basal SP4 (per 1L total SP4):

Difco PPLO Broth	3.5 g	Difco/Per 1L: Beef heart infusion from 50 g – 6 g Peptone – 10 g NaCl – 5 g
Tryptone (peptone from casein)	10 g	Sigma Aldrich
Peptone (peptone from gelatin)	5.3 g	Sigma Aldrich

Add 300 ml H2O and autoclave – the powders will not fully dissolve at this concentration in water, the autoclaving is required
Just make sure that there are no visible clamps of powder in the solution, just uniform ‘paste’ of particles. Autoclaving will yield a homogenous, dark-yellow solution.
Do not add basal SP4 powders directly to the rest of SP4 recipe! Dissolving these powders without autoclaving will be a challenge.

If make serum-free SP4, replace FBS with milliQ H2O.
pH to 7.0
Heat-inactivate for 30 min at 56°C.
Keep at 4°C.