

Recipes

Note: We and others have simplified the protocol described in Samuels (Methods in Molecular Biology, 1995, **47**:253-259), by combining a few of the components.

1.5x BSK⁺ (for plates)

BSA (Millipore Probumin 81-003, see note on testing)	69.4 g/l
Neopeptone (BD 211681)	6.9 g/l
Hepes acid (Calbiochem 391338)	8.3 g/l
Glucose (Sigma G-7021)	6.9 g/l
Sodium citrate (Sigma C-7254) (or citric acid)	1.0 g/l
Sodium pyruvate (Sigma P-5280)	1.1 g/l
N-acetyl glucosamine (Sigma A-3286)	0.6 g/l
Sodium bicarbonate (Sigma S-3817)	6.4 g/l
Yeastolate (BD 255772)	3.5 g/l
10x CMRL powder (USBiological C5900-5)	12.7 g/l

We typically make batches of 5l. Solubilize BSA in full volume of distilled water. Add rest of ingredients and stir gently until solubilized (can take 2-4 hr). Adjust pH to 7.5 with NaOH. Filter sterilize, ideally using a peristaltic pump to provide positive pressure to a 0.2 mm filter (e.g., Nalgene Fast PES filter 5609-0020). Aliquot 300 ml into 500 ml sterile bottles, add 12 ml rabbit serum (Pel-Freez 31125), and freeze.

BSKII

10x CMRL	9.7 g/l
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Neopeptone	5 g/l
BSA	50 g/l
Yeastolate	2 g/l
Hepes acid	6 g/l
Glucose	5 g/l
Sodium citrate	0.7 g/l
Sodium pyruvate	0.8 g/l
N-acetyl glucosamine	0.4 g/l
Sodium bicarbonate	2.2 g/l
Gelatin	10 g/l

Mix first 3 ingredients – stir until dissolved. Add other ingredients. Gelatin should be dissolved bit-by-bit in boiling water while stirring, then added. Adjust pH to 7.6, then place flask in 55°C until ready to filter. Filter through 0.2 mm filter (e.g., Nalgene Fast PES filter 5609-0020). Aliquot 500 ml into bottles. Add 30 ml rabbit serum per bottle and store frozen. We generally aliquot 500 ml bottles into 100 ml bottles after thawing, and refreeze bottles that are not in use.

Bb antibiotics (100 X)

2 mg/ml phosphomycin

5 mg/ml rifampicin

250 mg/ml amphotericin B

solubilized in 20% DMSO.

Testing medium components

We always test BSA as follows. Call Millipore and ask for samples (at least 100 g) of two or three lots containing at least 5 kg and have the remainder held. Prepare a small batch of 1.5x BSK+ from each lot. Compare time for colony formation and colony morphology of your favorite low passage strain (we usually use B31 A3) for each batch to the same parameters for your current batch. Choose a lot that is at least as good as your current lot and buy at least 5 kg. Rabbit serum can be tested in the same manner, if using a new supplier or if there is a suggestion of a problem.

EPS

Sucrose 93.1 g/l

Glycerol 150 g/l

Filter through 0.2 mm filter. Keep in refrigerator.