Growth of bloodstream and procyclic *T. brucei* colonies on agarose plates

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The original protocol, devised by Vern Carruthers, was **simplified by Kevin Tan (2002: see last section of this document)**, avoiding the need to prepare 2X concentrations of the liquid medium.

Introduction (Vern)

Growth of procyclic (PF) colonies has been described (Lee & van der Ploeg, 1989), but the method was limited by extremely low plating efficiencies (0.01%). The technique described here (Carruthers & Cross, 1992) is a modification of the Lee and van der Ploeg method, using SDM-79 agarose plates containing conditioned medium to increase the plating efficiency dramatically for PF. By substituting HMI-9 (Hirumi & Hirumi, 1989) for SDM-79, bloodstream forms (BF) can also be grown as colonies. In my hands, HMI-9 medium works very well for adaptation and growth of bloodstream forms in liquid culture (see separate protocol). I have successfully grown three variants from the 427 strain and one S42 (pleomorphic) strain variant as colonies on plates. However, it has not yet been determined if all strains and variants can form colonies. If you do not require a specific variant, I suggest you use MITat 1.2 (221) since it appears to grow the best. Colonies can be lifted onto filters and processed according to standard protocols for hybridization with DNA probes or possibly antibodies (not tested).

Vern's original protocol

(A) Preparation of 2X HMI-9—Dissolve powdered IMDM (Gibco cat. # 430-2200EB) and $NaHCO_3$ in the stated volume of water and add the supplements (see elsewhere for supplement recipes). Sterilize by filtration through an 0.22 μm membrane and place in a 50 °C bath.

Ingredient	5 plates	10 plates	15 plates	20 plates
Powdered IMDM	1.32 g	2.66 g	3.98 g	5.31 g
$NaHCO_3$	0.227 g	0.454 g	0.68 g	0.907 g
Water	23 ml	46 ml	69 ml	94 ml
100X Bathocuproine sulfonate	1.0 ml	2.0 ml	3.0 ml	4.0 ml
100X Cysteine	1.0 ml	2.0 ml	3.0 ml	4.0 ml
100X 2-ME	1.0 ml	2.0 ml	3.0 ml	4.0 ml
100X Thymidine	1.0 ml	2.0 ml	3.0 ml	4.0 ml
100X Pyruvate	1.0 ml	2.0 ml	3.0 ml	4.0 ml
100X Pen/Strep	1.0 ml	2.0 ml	3.0 ml	4.0 ml
FBS	10 ml	20 ml	30 ml	40 ml
Serum Plus	10 ml	20 ml	30 ml	40 ml

(B) Preparation of 2X SDM-79—Dissolve powdered SDM-79 (JRH Biosciences cat. # 57-453-165) in the given amount of H_2O then add FBS, conditioned medium (grow procyclics to late log phase, spin and carefully remove supernatant) and hemin (2.5 mg/ml in 50 mM NaOH).

Ingredient	5 plates	10 plates	15 plates	20 plates
powdered SDM-79	1.83 g	3.67 g	5.49 g	7.33 g
Water	20.7 ml	41.4 ml	62.1 ml	82.8 ml
FBS	8.0 ml	16.0 ml	24.0 ml	32.0 ml
Conditioned Medium	20 ml	40 ml	60 ml	80 ml
Hemin	288 μl	576 μl	864 μl	1152 μl

Adjust pH to 7.4 by adding 10 N NaOH in 50 µl aliquots. Pre-filter through a 0.4 µm membrane (if available), then sterilize using a 0.22 µm membrane and place in a 50 °C water bath.

(C) Preparation of Agarose

Prepare a suspension of low melting temperature agarose (Type VII, Sigma Cat. # A4018) in a tissue culture media bottle. Autoclave 15 min, mix thoroughly and place in a 50 °C water bath.

Ingredient	5 plates	10 plates	15 plates	20 plates
Agarose 0.65 g	1.30 g	1.95 g	2.60 g	
Water	50 ml	100 ml	150 ml	200 ml

Once the medium and agarose solutions have equilibrated to 50 °C, mix them thoroughly and dispense 20 ml volumes into each 10 cm Petri dish in a sterile hood and allow at least 30 min to set. Plates can be used immediately or stored upright at 4 °C for at least 2 months.

Pre-dry plates for exactly 1 h in a sterile hood (vertical laminar flow) with lids off.

Count cells then dilute to desired density and spread 100 µl of cell suspension per plate using a glass rod sterilized by flaming in alcohol (be sure to allow enough time for cooling to RT). Allow the plates to dry again for 10 min.

For HMI-9 plates, place in 37 °C/5% CO_2 incubator for several hours to O/N to allow pH equilibration before sealing edges with parafilm. SDM-79 plates can be sealed and placed at 27 °C immediately. It doesn't seem to matter whether the plates are inverted or not.

You can expect to see BF colonies after incubation for 3.5–5 days. They should appear as slightly blurry opaque white growths 1–3 mm in diameter consisting of about 10^6 cells at maturity. Plating efficiency should be in the 10–70% range (depending on the variant and strain). Procyclic colonies take approximately 2 weeks to become visible and plating efficiency of about 15–20% can be expected.

Kevin Tan (2002) modifications simplify plate preparation

Dissolve 6.5 g of agarose in 100 ml water and autoclave. Equilibrate the melted 6.5% agar solution and 200 ml of normal strength HMI-9 to 50 °C in a water bath. Mix 10 parts of medium with one part of 6.5% agarose and pour 22 ml aliquots into sterile Petri dishes. Allow to set for 30 min at room temperature or overnight at 4 °C. Before use, dry the plates in a sterile hood, with lids off, for exactly 1 h. Dilute BF *T. brucei* to 10^3 cells/ml in HMI-9, pipette 500 µl aliquots onto each plate and disperse evenly using a glass spreader. Dry the plates, with lids off, for 10 min and incubate overnight in a 37 °C humid CO_2 incubator, to ensure pH equilibration before sealing individual plates with Parafilm to prevent drying. Colonies become visible to the unaided eye around day 5.