Trypanosome Media Preparation Protocols

IMPORTANT: the web-page html version is considered definitive and may have minor corrections that are not in this document, although I will attempt to keep the two versions congruent.

Please note that we do not add antibiotics to any media: we do routinely test an aliquot for sterility after filtration.

HMI-9 — For 10 liters for bloodstream forms

8 l	IMDM (Invitrogen/GIBCO Cat. No. 12440)
1 l	FBS (heat-inactivated at 55 °C for 1 h)
1 l	Serum Plus™ (JRH Biosciences Cat. No. 14001)
100 ml	Hypoxanthine stock (dissolve 4.0 g of NaOH in 1 l water, add 13.6 g hypoxanthine and freeze in 100 ml aliquots).
280 mg	Bathocuproine disulfonic acid (final concentration is 50 μm)
1,820 mg	Cysteine (add after Bathocuproine) (final concentration is 1.5 mM)
1,100 mg	Pyruvic acid
390 mg	Thymidine
140 μl	2-mercaptoethanol

Filter-sterilize and store in 500 ml bottles at 4 °C.

SDM-79 minus $NaHCO_3$ — For 10 liters for procyclic forms

Note: SDM used to be available from JRH Biosciences Inc, prepared to order, Cat. No. 57453, but may not be available since 2005, when JRH was incorporated into Sigma. A revised formulation on 12th November 1998 incorporated all components individually, resulting in some duplications of the previous hybrid formulation, and omits sodium bicarbonate. We'll try to reformulate to eliminate duplications on the next order.

Add 1 bottle (254.7 g) of JRH powder to 9 liters of water and adjust the pH to 7.4 with ~ 35 ml of 10 M NaOH. Filter sterilize 450 ml portions into 500 ml bottles and freeze. Before using, add 1.5 ml hemin stock solution (final concentration will be 7.5 mg/l) and 50 ml heat-inactivated serum to 450 ml medium.

The hemin stock solution is prepared by dissolving 400 mg of NaOH in 200 ml water and adding 500 mg hemin. Autoclave for 20 min and store as 20 ml aliquots at 4 °C.

DTM — for 1 liter for differentiation of bloodstream to procyclic forms*

A key feature of this medium is the absence of glucose. Because MEM minus glucose is not commercially available, DTM is a bit more tedious to prepare. IMDM is available without glucose, but its amino acid composition differs significantly from MEM. DTM calls for adding BME vitamins solution, which GIBCO (Invitrogen) no longer supplies, for lack of demand. The

formulation of MEM vitamins (100x, Invitrogen/GIBCO product number 11120) is identical to BME except for the absence of Biotin, which must now be added separately.

* Synchronous differentiation of Trypanosoma brucei from bloodstream to procyclic forms in vitro. Ziegelbauer K, Quinten M, Schwarz H, Pearson TW & Overath P (1990) Eur J Biochem 192:373-8. There is an error in methods in this paper, where modified DTM refers to 10 mM glycine instead of glycerol. The original DTM was further modified (as in the protocol given here) by adding glycerol (in place of glucose), heme, 15% fetal bovine serum, 0.2 mM 2–mercaptoethanol, and extra proline, glutamate, glutamine. Vassella and Boshart further modified DTM by adding 28.2 mg/l bathocuproine and 182 mg/l cysteine (Vassella E, Boshart M. High molecular mass agarose matrix supports growth of bloodstream forms of pleomorphic Trypanosoma brucei strains in axenic culture. Mol Biochem Parasitol 1996;82:91-105). You might want to consider doing this and adding the same two ingredients to SDM79, or any other medium you use.

For 1 liter (nominal final volume), add the following to 900 ml water, adjust the pH to 7.2 with 5 M NaOH and filter-sterilize.

6,800 mg	NaCl
400 mg	KCl
200 mg	$CaCl_2$
140 mg	$NaH_2PO_4.H_2O$
200 mg	$MgSO_4.7H_2O$
7,940 mg	HEPES
2,200 mg	$NaHCO_3$
110 mg	Sodium pyruvate
10 mg	Phenol Red
14 mg	Hypoxanthine
1.0 mg	Biotin
760 mg	Glycerol (1.21 ml of a 50% solution of glycerol)
640 mg	Proline
236 mg	Glutamic acid
1,340 mg	Glutamine
3 ml	Hemin (stock solution at 2.5 mg/ml as described above for SDM79)
20 ml	50x MEM amino acids solution (Invitrogen/GIBCO Cat. No. 11130)
10 ml	100x MEM non-essential amino acids solution (Invitrogen/GIBCO Cat. No. 11140)
10 ml	100x MEM vitamin solution (Invitrogen/GIBCO Cat. No. 11120)
14 µl	2-mercaptoethanol (final concentration will be 0.2 mM)
150 ml	Heat-inactivated FBS