

Preparation of Hemin Solution

1. To make 1mM hemin solution, dissolve 3.26 mg hemin (Hemin Fluka, Sigma51280, FW 652.0) in 5 ml 20mM NaOH.
2. Dilute above hemin solution to 10 uM by taking 100 ul of 1 mM hemin solution to 10 ml S-MEM pre-warmed at 37 C.

Protocol for Hemin Treatment

1. Culture cells for 36 hours (be sure to give cells enough time from them to express the protein of interest). Note that the condition of culture is upon the type of cells.
2. Remove the medium, wash cells twice with warmed S-MEM.
3. Add 1 ml of 10 uM hemin solution into the cells and continue to incubate at 37 C for 3-4 hours.
4. Remove the hemin and wash the cells twice with 1x HBSS. Add the proper lysis buffer.

S-MEM: Minimum Essential Medium

HBSS: Hanks' Balanced Salt Solution

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methemalbumin = Hemin-albumen complex

Ref: Appleton et al, 2003. Effects of hypoxia on heme oxygenase expression in human chorionic villi explants and immortalized trophoblast cells *Am J Physiol Heart Circ Physiol* 284 Vol. 284, Issue 3, H853-H858.

The stock solution of methemalbumin (1.5 mM hemin and 0.15 mM BSA) was prepared as previously described ([35](#)). Briefly,

Hemin was dissolved in 0.5 ml of aqueous 10% (wt/vol) ethanolamine. (9.8 mg/0.5 ml PBS- make up in 16 ml tube- Just made up in PBS)

BSA dissolved in 2 ml of deionized water was added to the hemin solution (BSA mw 67,000- 100 mg/2 ml).

The volume was made up to 7 ml and slowly adjusted to pH 7.4 with 1 M HCl and vigorous stirring. (pH was ~6.8- very difficult to titrate, so did not attempt to)

The final volume for the stock solution was adjusted to 10 ml with deionized water.

The methemalbumin stock solution was prepared with the laboratory lights turned off and was stored at -20°C for up to 1 month. (Ron lab keeps at 4 degrees)

Alt 2:

Hemin (Fluka, 1 ml of 12.4 mM stock in 0.1 N NaOH) was added to 4 ml of 10 mM phosphate buffer, pH 7, containing 8 nmol of BSA (Sigma) to give a 1.5 molar ratio of hemin to albumin.