Preparation of Myocyte Lysates for Cyclic AMP Determination
AfCS Procedure Protocol PP00000131
Version 1, 03/06/03

The method chosen for measuring the content of cyclic adenosine 3',5'-monophosphate (cyclic AMP or cAMP) in cardiac myocytes is an enzyme-linked immunoassay system developed by Amersham Biosciences. An overview of the assay, cAMP Biotrak EIA, and its instructions, Biotrak Protocol, are available through the indicated links. The procedure described below provides a sufficient sample for several determinations of cAMP using the protocol, Assay of Cyclic AMP in Lysates of Cells (AfCS Procedure Protocol ID PP00000015).

Treatment of Cells and Preparation of Extracts
1. For each desired sample, plate 50,000 rod-shaped cells in one 35-mm dish or each well of a 6-well plate (growth area 9.5 cm²), and culture with 1 ml of myocyte culture medium (MC culture medium).
2. Culture in an incubator with 2% CO₂ at 37 °C for 18 to 22 hr.
3. Transfer the dishes to an environmental chamber (see Use of Environmental Chamber, below) at 37 °C and 2% CO₂.
4. Begin treatments at timed intervals by addition of 0.5 ml of ligand or vehicle (3X final concentration in MC culture medium) to appropriate wells. (Note: vehicle controls constitute matching dilutions of solvents in which ligands are dissolved and stored.)
5. Mix with a microtiter plate shaker for 5 sec at 200 rpm.
6. Incubate at 37 °C in the environmental chamber with 2% CO₂. Exception: if the 2% CO₂ environment is not available and the time of exposure to ligand is more than 5 min, place cultures in incubator at 37 °C with 2% CO₂ for the incubation with ligand.
7. At the desired times, end treatments with the addition of 3 ml of 100% ethanol (final concentration of 65% ethanol); mix by tilting dishes or plates in a circular motion.
8. After all treatments on a plate have been terminated, place the plate on ice for 5 min.
9. Transfer lysates to labeled, 15-ml conical tubes.
10. Add 1 ml of 65% ethanol to the same wells and mix by tilting the plate in a circular motion to rinse the wells.
11. Add the rinses to the matching samples in the 15-ml conical tubes; cover the tubes and mix by inversion.
12. Centrifuge the tubes at 2000 x g for 15 min at 4 °C.
13. Transfer a 1-ml aliquot of each supernatant (1/4 of the total volume, or the equivalent of 10,000 to 12,000 rod-shaped cells) to barcoded 1.5-ml Eppendorf tubes.
14. Dry the samples in a vacuum (Speed Vac) with centrifugation and heat until the samples are completely dry (about 2 to 3 hr).
15. Secure caps on the samples and store at –80 °C until determination of cAMP by enzyme immunoassay (EIA). Note: samples are dissolved in 100 µl of water prior...
Use of Environmental Chamber
An environmental chamber with temperature control is used to maintain uniform
temperature during experimental treatments. High humidity is maintained with an open
tray of water in the chamber. When available, CO₂ is maintained at 2% by manual
injection of 100% CO₂, as required, and monitoring with a CO₂ sensor. Note: long-term
cultures (overnight) are only maintained in standard tissue culture incubators.

Reagents and Materials
Tissue culture dish, 35 mm: Falcon; catalog no. 3001
Tissue culture plate, 6 well: Falcon; catalog no. 3046
Myocyte culture medium (MC culture medium): AfCS Solution Protocol ID PS00000446
Incubator: NAPCO; catalog no. 51201068
Environmental chamber (aluminum glove box with temperature control and pass-
through airlock): Coy Laboratory Products; catalog no. 0850-003
Microtiter plate shaker: VWR International; catalog no. 57019-600
Ethanol, 100%: Aaper Alcohol and Chemical Co.; catalog no. 030801
Conical tubes, 15 ml: Greiner; catalog no. 188261
Ethanol, 65%: AfCS Solution Protocol ID PS00000075
Microfuge tubes, 1.5 ml: SARSTEDT; catalog no.72.690
Speed Vac System: Savant; catalog no. SS21
CO₂ sensor: TSI Inc.; model no. 8560

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