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ATP Extraction & Measurement

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Mariano MP Marin-Blazquez[1]
[1]UCAM

Mariano MP Marin-Blazquez
Universidad Católica San Antonio de Murcia

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for extracting and quantitatively measuring ATP content in cultured cells

Materials

- EDTA
- TCA
- mQ H_2O
- Material needed for cell trypsinization
- PBS
- Trypsin
- DMEM medium
- 15 ml Falcon tubes
- Serological pipettes
- Ice
- ATP Determination Kit from Invitrogen
- White opaque 96-well microplates with opaque bottoms
- Microplate luminescence reader

Troubleshooting

Safety warnings

The Standard Reaction Solution must always be protected from light. After every sample is prepared, the plate must be loaded in the dark to avoid luciferin degradation. This protocol is designed to be used with cell samples coming from at least a T-25 flask. Lower cell numbers may result in undetectable ATP amounts.

ATP Extraction

1 Prepare 1% (w/v) TCA by diluting 1 g of TCA for each 100 ml of mQ H_2O

2 Trypsinize cells and resuspend in 1 mL of PBS

3 Add 500 µL/sample of 1% cold TCA

4 Vortex

5 Incubate for 10 min on ice

6 Centrifuge at 12,000 g for 10 minutes

Reagent preparation

7 If using the kit for the first time: Prepare a 100 mM DTT stock solution by adding 1,62 mL of H_2O mQ to the bottle containing 25 mg DTT. Aliquot into ten 160 µL volumes and store frozen at ≤20 ºC. Stock solutions of DTT are stable for six months to one year. Thawed aliquots should be kept on ice or at 4 ºC until ready to use

8 Make 1 mL of 1X Reaction Buffer by adding 50 µL of 20X Reaction Buffer to 950 µL of H_2O mQ

9 Make 1 mL of 10 mM of luciferin solution by adding 1 mL of 1X Reaction Buffer to one vial of luciferin (blue cap). **Protect from light until use**. This stock solution is reasonably stable for several weeks if stored at ≤ 20 ºC, protected from light. Divide in 250 µL aliquots

10 Prepare a 10 mL Standard Reaction Solution, which will be enough for one microplate as follows:

10.1 8.9 mL H_2O mQ

10.2 0.5 mL 20X Reaction Buffer

10.3 0.1 mL 100mM DTT

10.4 0.5 mL 10 mM luciferin

10.5 2.5 µL luciferase

11 Gently invert the tube to mix, **do not vortex**. **Keep the reaction solution protected from light until ready to use**. Although the solution may be stored at 2-6 ºC protected from light for several days, assay sensitivity will diminish with time.

ATP standard curve preparation

12 Prepare a 100 µM ATP stock solution by adding 1 µL of 5 mM ATP standard solution to 49 µL of H_2O mQ (total volume: 50 µL)

13 Prepare the following dilutions

13.1 10 µM 10X. Add 10 µL of the stock solution to 90 µL of H_2O mQ

13.2 1 µM 10X. Add 10 µL of the previous solution to 90 µL of H_2O mQ

13.3 0.5 µM 10X. Add 50 µL of the previous solution to 50 µL of H_2O mQ

13.4 0.25 µM 10X. Add 50 µL of the previous solution to 50 µL of H_2O mQ

13.5 0.1 µM 10X. Add 40 µL of the previous solution to 60 µL of H_2O mQ

13.6 0.05 µM 10X. Add 50 µL of the previous solution to 50 µL of H_2O mQ

13.7 0.025 µM 10X. Add 50 µL of the previous solution to 50 µL of H_2O mQ

14 To the microplate, add two to three duplicates of standard reaction solution as a blank measure of background luminescence

15 Add two to three duplicates of each 1X dilution to the microplate, adding 10 µL of the dilution and 90 µL of Standard Reaction Solution

ATP measurement

16 Prepare several serialized dilutions for ATP determination of the sample

17 Add 10 µL of the serialized dilutions of the sample and 90 µL Standard Reaction Solution, taking into account that this is also a 1:10 dilution on the microplate

18 Incubate for 10-15 minutes at room temperature and protected from light

19 Measure at 560 nm, remove background signal and standardize using the Standard curve