Protocol Title

ATP determination with ROCHE kit
“ATP Bioluminescence Assay Kit CLS II”

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Notes
Protocol is adapted for ATP determination in yeast in 96 well plate-format from manual for ROCHE “ATP Bioluminescence Assay Kit CLS II”, Cat #: 1 699 695

Abstract:

The kit is used for the quantitative detection of ATP in yeast cells by luciferase driven bioluminescence. ATP extraction is done by boiling cells in TE buffer.

Materials:

- Luciferase reagent – follow instructions in the manual
- ATP standard (16.5 mM) – follow instructions in the manual
- TE7.75 – 100 mM Tris (pH 7.75), 4 mM EDTA
- LumiNunc F96 Microwell Plates (Nunc, Cat # 236107)

Equipment:

- Multi-well plate reader (Wallac, Victor² Multilabel Counter)
- 100°C water bath or thermo block
- Eppendorf tube centrifuge

Procedure:

1. Mix 50 µl of cell suspension (cell concentration between 1x10⁷ and 1x10⁸ cells/ml) with 450 µl of boiling TE7.75
2. Incubate for 2 min at 100°C
3. Centrifuge sample at 1000 x g for 1 min
4. Transfer 50 µl of the supernatant to a 96 well plate (and keep on ice until measurement)
5. Dilute ATP standard to the following dilutions: 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹, 10⁻¹⁰ M; transfer 50 µl per dilution to the 96 well plate
6. Add 50 μl of Luciferase reagent to sample (or standards)
7. Remove from ice, mix and let stand 15 min at room temperature
8. Mix and measure luminescence in plate reader (3 seconds integration time, measurement height approx. 8 mm)
9. For the calculation of ATP concentrations per cell culture concentration and average cell size were determined with a cell counter (CASY1, Schärfe System)