Promega

Product Application

RNA extraction from *S.cerevisiae*

Isolate high quality, amplifiable RNA from S.cerevisiae were extracted using ReliaPrep™ RNA Cell Miniprep System

Kit: ReliaPrep™ RNA Cell Miniprep System (Cat.#Z6012)

Analyses: GoTaq® 1 step RT-qPCR, QuantiFluor® RNA system and NanoVue™ quantitation

Sample Type(s): *Saccharomyces cerevisiae*

Input: up to $9*10^7$ cells

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM370, available at: **www.promega.com/protocols**

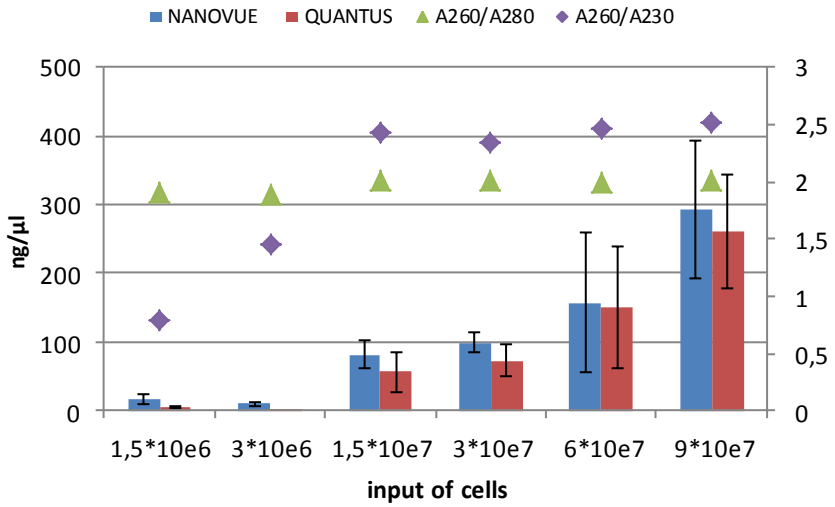
Materials Required:

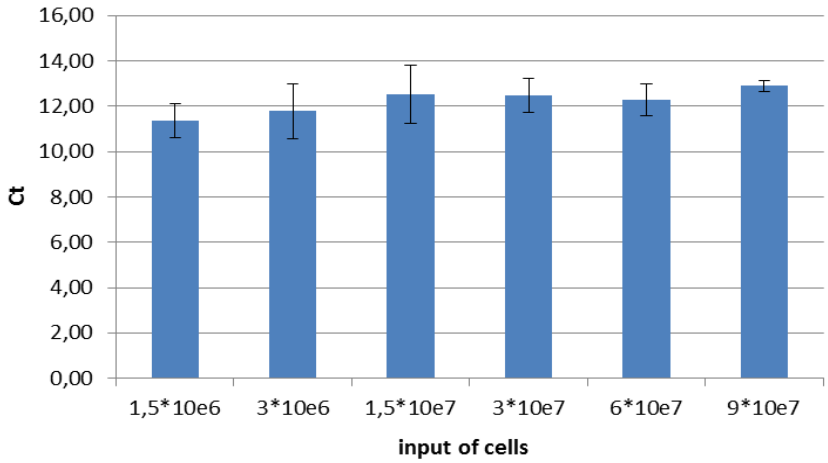
- ReliaPrep™ RNA Cell Miniprep System (Cat.#Z6012)
- Lyticase at 4 units/µl
- D lysing matrix tubes for use with MP Bio FastPrep®-24 Instrument (Ref. 6913-100)
- Thermomixer

Protocol:

1. Grow a culture of *S. cerevisiae* overnight at 30°C in YPD medium.
2. Make a 1:10 dilution of the overnight culture. Grow the culture to an OD_{600} approx. 0.8-1 (around 16h at 30°C and 600 rpm). An OD~1 has $3*10^7$ cells of yeast.
3. Put up to $9*10^7$ in a 1.5 ml tube and make the pellet.
4. Add 12.5 µl of lyticase and 87.5 µl TE X1 to each tube. Incubate 60 min at 30°C and at 1000 rpm in a thermomixer.
5. Add 250 µl BL+TG buffer and vortex 5 sec.
6. Do the beads treatment: 6 m/s during 40x2 sec.
7. Continue with standard protocol in the technical manual (TM370).

Results:





Top. RNA concentrations based on quantitation using the Quantus™ instrument with QuantiFluor® RNA System (Cat. #E3310) and NanoVue. (n=3) **Bottom:** Ct values were determined using GoTaq®1-Step RT-qPCR System (Cat. # A6020), 18S primers[1] and 5 ng of RNA extracted in 50 µl of reaction (n=2).

Reference:

1. Cabib, E., Roh, D.H., Schmidt, M., Crotti, L.B. & Varma, A. The yeast cell wall and septum as paradigms of cell growth and morphogenesis. J Biol Chem 276, 19679-19682 (2001).