Ribosomes play an important role as a site of protein synthesis inside cells. The structure and the mechanisms of action have been investigated. Ribosome is an RNA-protein complex. It is comprised of a large subunit and a small subunit. There is a 70S ribosome that is comprised of a 30S subunit and a 50S subunit in prokaryotic Escherichia coli.

Following is our experiment report on separation of 70S ribosomes from Escherichia coli by means of the newly developed S50ST swinging bucket rotor that can hold the 7PA tubes and has the largest capacity in its class.

Experiment

1. Instruments
   - Centrifuge: CS150NX tabletop micro ultracentrifuge
   - Rotor: S50ST swinging bucket rotor (Up to 4 tubes can be contained.)
   - Tube: 7PA tube (Actual capacity: 7 ml)

2. Separation procedure
   - Suspend Escherichia coli in TMAI buffer and mix it with glass beads to crush. Perform a quick centrifugation to remove the glass beads.
   - Perform centrifugation using the S50ST swinging bucket rotor. (39,000 rpm, 30 minutes, 4°C)
   - Remove the supernatant and perform centrifugation using the S50ST swinging bucket rotor. (34,000 rpm, 6 hours, 4°C)
   - Suspend the sediment (crude 70S ribosome fraction) in 1.8 ml TMAI buffer.
   - Layer it on 5 ml of 30% (W/V) sucrose – TMAI buffer.
Perform centrifugation using the S50ST swinging bucket rotor. (40,000 rpm, 15 hours, 4°C)

Suspend the sediment (purified 70S ribosome) in 2 ml TMA buffer and store it at -70°C.


Instrument

CS150NX tabletop micro ultracentrifuge

S50ST swinging bucket rotor

*Easy-to-set top loading type bucket

*This rotor can also be used with the CS150NX, CS-GX II series and CS-GXL series centrifuges.

If you have any inquiry of this application or products, please contact us through our web site.

http://www.hitachi-koki.com/himac.contact/index.htm

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