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himac APPLICATION

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Experiment on Percoll® density gradient centrifugation by means of 50-ml conical tubes

R18A fixed angle rotor for high-speed refrigerated centrifuge

Feasibility of separating cell organelles by means of Percoll®

Amersham Biosciences' Percoll® is a very convenient density gradient medium for centrifugation that eliminates operators having to prepare density gradients before centrifugation. However, the density gradient centrifugation using such a self-forming medium requires an RCF at least 30,000 x g, therefore ultracentrifuges have been used for the density gradient centrifugation generally. Although the 50-ml conical tube is very popular in the bio-related industries, it cannot be used for the density gradient centrifugation because it cannot bear the RCF required for the operation.
This time, we have developed a new 50-ml conical tube that can be used at the maximum speed 18,000 rpm and the maximum RCF 42,200 x g, and the angle rotor specifically designed for this tube. The new 50-ml conical tube is less expensive and more convenient than the conventional ultracentrifuge tubes. Following is our experiment result on feasibility of separating cell organelles by means of Percoll® with the new 50-ml conical tube and the angle rotor. In this experiment, 0.15M NaCl (saline) was added to the undiluted solution of Percoll® on the market, then five kinds of density gradient markers whose buoyant densities were from 1.017g/ml to 1.119g/ml were added. After mixing, the uniform solution was centrifuged. Formation of density gradients is compared with the case of centrifugation at 12,000 rpm (maximum speed of the conventional 50-ml conical tube) for study.

1. Equipment and experimental conditions

Centrifuge: CR-G series high-speed refrigerated centrifuge
Rotor: R18A fixed angle rotor
Tubes: himac 50TC tubes
Time: 30 minutes
Temperature: 20℃
Acceleration and deceleration: ACCEL mode "9", DECEL mode "5"
Density gradient medium: 67% (v/v) Percoll® (in 0.15M NaCl)
Buoyant density of the density gradient marker: 1.017, 1.034, 1.074, 1.088, and 1.119g/ml (five kinds)

2. Result and comparison

(1) Speed 18,000 rpm
Maximum RCF: 42,200xg

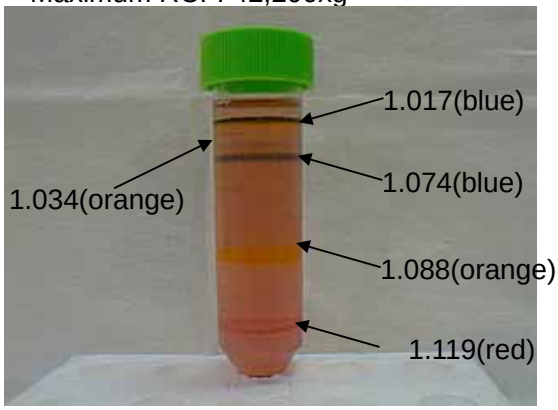

Fig.1

(2) Speed 12,000 rpm
Maximum RCF: 18,800xg

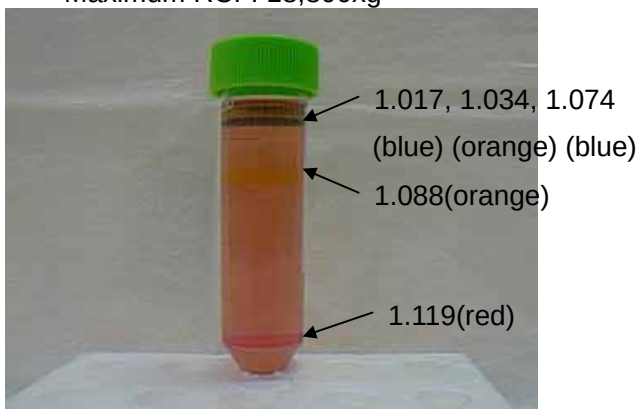

Fig.2

(Both Figs.1 and 2 show five layers 1.017(blue), 1.034(orange), 1.074(blue), 1.088(orange), and 1.119g/ml(red) from the top.)

3. Explanation

Percoll® was diluted to about 67% (v/v) with 0.15M NaCl, then proper amounts of five density gradient markers were added to it and mixed well. The suspension was centrifuged by means of the himac 50TC tube and the R18A fixed angle rotor at 18,000 rpm for 30 minutes. Figure 1 shows the result of the centrifugation. Although the separation between the top layer 1.017g/ml(blue) and the layer of 1.034g/ml(orange) under the top is insufficient, the layers of 1.074g/ml(blue), 1.088g/ml(orange), and 1.119g/ml(red) are separated sufficiently as shown in the photograph. Thus it shows the density gradients are sufficiently formed.

Figure 2 shows the result of the centrifugation at 12,000 rpm (maximum speed of the conventional 50-ml conical tube) for 30 minutes. The three layers of 1.017(blue), 1.034(orange), and 1.074g/ml(blue) are not separated. The layer of 1.088g/ml(orange) is at the higher position than the center of the tube and the layer of 1.119g/ml(red) is near the bottom of the tube. Thus the density gradient markers are widely dispersed from the top of tube to the bottom, and the density gradients are not formed sufficiently.

As a result of the experiment, it is expected that the following cell organelles can be separated by means of Percoll® thanks to the combination of the R18A angle rotor and the himac 50TC tube.

Nuclei (1.09), mitochondria (1.10), lysosomes (1.06), peroxisomes (1.06), plasma membranes (1.03)
(The buoyant densities of each cell organelle in Percoll® are indicated in parentheses (unit: g/ml).)

In addition, the layer of 1.017g/ml can be sufficiently separated from the layer of 1.034g/ml by decreasing the initial density of Percoll®.

4. Percoll®

Percoll® is a medium for density gradient centrifugation that is marketed by Amersham Biosciences. It is the colloidal silica coated with polyvinylpyrrolidone (PVP). It can be used for separation of cells, intracellular granules (cell organelles) and large viruses, and also for formation of density gradients up to 1.3g/ml. It has no cytotoxicity.

Physical property is as follows.

Density (g/ml): 1.130±0.005
Conductivity (mS/cm): ＜1.0
Osmolality (mOs/kgH2O): ＜25
Viscosity (cP): 15 at 20℃
pH 9.0±0.5 at 25℃

Percoll® is a registered trade name of Amersham Biosciences.

For further information, please contact the personnel in charge of application in Life-Science Instruments Division, Hitachi Koki Co., Ltd. (Fax: +81-3-3226-7845).

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