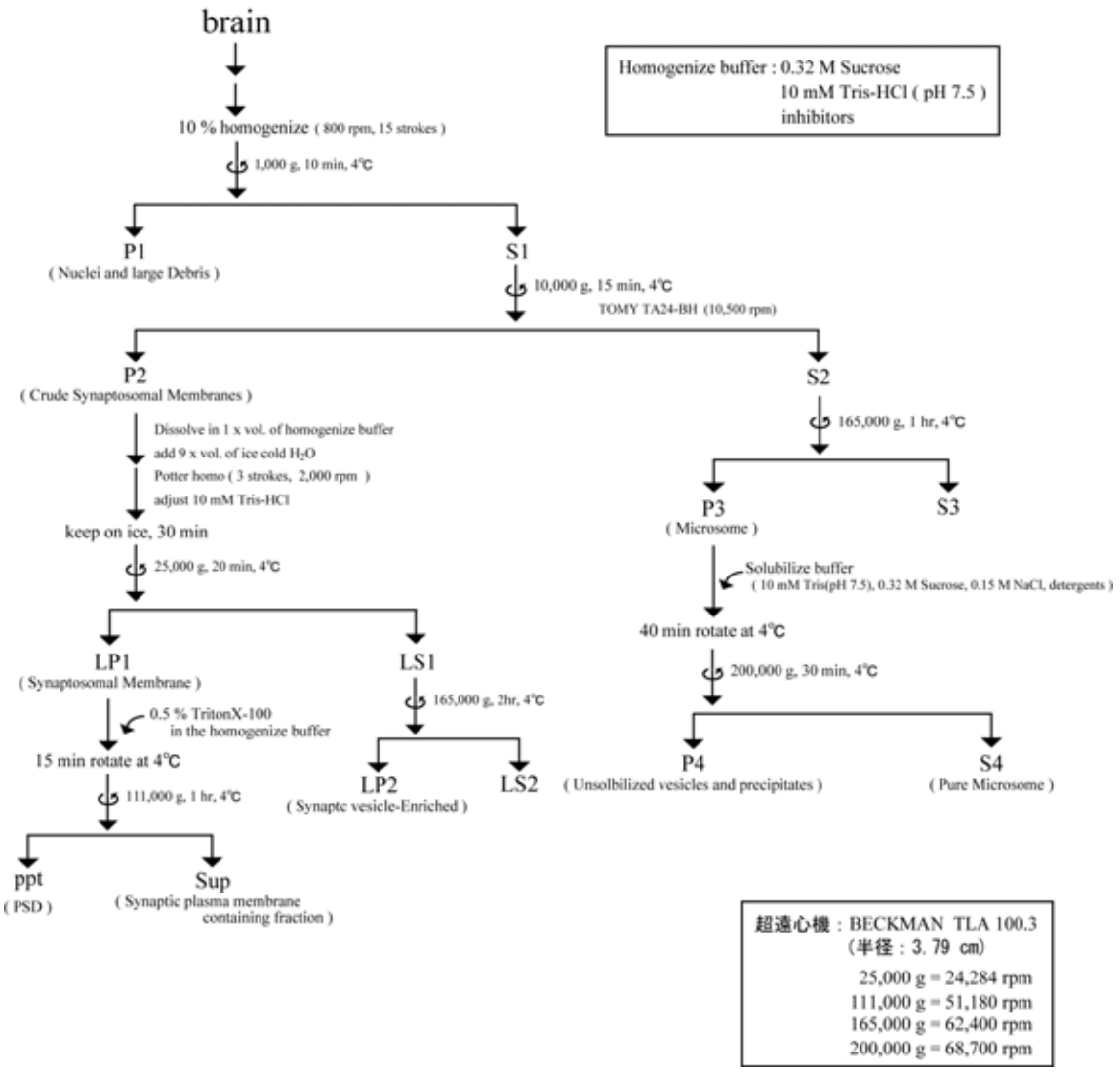
細胞分画法


brain
Homogenize buffer : 0.32 M Sucrose
10 mM Tris-HCl (pH 7.5)
inhibitors
10 % homogenize (800 rpm, 15 strokes)
1,000 g, 10 min, 4℃
P1
(Nuclei and large Debris)
S1
10,000 g, 15 min, 4℃
TOMY TA24-BH (10,500 rpm)
P2
(Crude Synaptosomal Membranes)
S2
Dissolve in 1 x vol. of homogenize buffer
add 9 x vol. of ice cold H2O
Potter homo (3 strokes, 2,000 rpm)
adjust 10 mM Tris-HCl
keep on ice, 30 min
25,000 g, 20 min, 4℃
165,000 g, 1 hr, 4℃
P3
(Microsome)
S3
Solubilize buffer
(10 mM Tris(pH 7.5), 0.32 M Sucrose, 0.15 M NaCl, detergents)
40 min rotate at 4℃
200,000 g, 30 min, 4℃
LP1
(Synaptosomal Membrane)
LS1
165,000 g, 2hr, 4℃
0.5 % TritonX-100
in the homogenize buffer
15 min rotate at 4℃
111,000 g, 1 hr, 4℃
LP2
(Synapte vesicle-Enriched)
LS2
P4
(Unsolbilized vesicles and precipitates)
S4
(Pure Microsome)
ppt
(PSD)
Sup
(Synaptic plasma membrane
containing fraction)
超遠心機 : BECKMAN TLA 100.3
(半径 : 3. 79 cm)
25,000 g = 24,284 rpm
111,000 g = 51,180 rpm
165,000 g = 62,400 rpm
200,000 g = 68,700 rpm