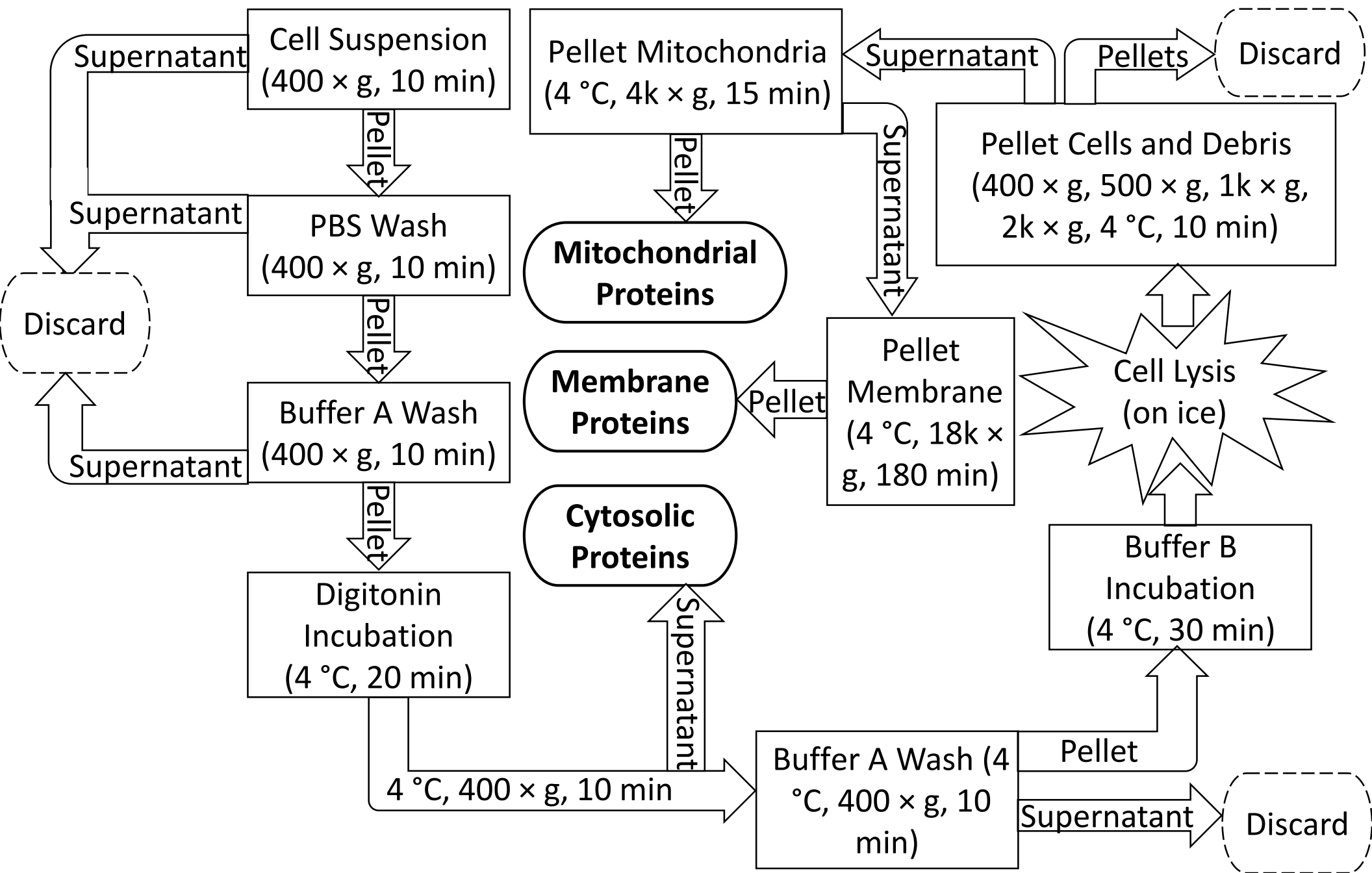
Cell Suspension (400 × g, 10 min)
Supernatant
Pellet
PBS Wash (400 × g, 10 min)
Supernatant
Discard
Pellet
Buffer A Wash (400 × g, 10 min)
Supernatant
Pellet
Digitonin Incubation (4 °C, 20 min)
4 °C, 400 × g, 10 min
Pellet Mitochondria (4 °C, 4k × g, 15 min)
Supernatant
Pellets
Discard
Pellet
Supernatant
Mitochondrial Proteins
Pellet Cells and Debris (400 × g, 500 × g, 1k × g, 2k × g, 4 °C, 10 min)
Membrane Proteins
Pellet
Pellet Membrane (4 °C, 18k × g, 180 min)
Cell Lysis (on ice)
Cytosolic Proteins
Supernatant
Buffer B Incubation (4 °C, 30 min)
Buffer A Wash (4 °C, 400 × g, 10 min)
Pellet
Supernatant
Discard