Isoflavones are known for their benefits for human health and they are in glycoside forms in vegetables. β-glucosidase catalyzes the hydrolysis of terminal non-reducing sugars releasing glucose and have high specificity for isoflavones. The aim of this work was to separate a soybean endogenous β-glucosidase from cultivar BRS 257. Enzyme was extracted with phosphate buffer 0.05M, pH 6.0 at 4°C from soybean cotyledons, (1:10, p.v) cultivar BRS 257. After centrifugation (15min, 15000g, 4°C), the supernatant was acidified to pH 5.0 with HCl 0.2N and centrifugated again. The crude extract was taken to fractionation with (NH₄)₂SO₄ at 0-40% saturation at 4°C. After new centrifugation, the supernatant fraction 0-40% was fractionated with (NH₄)₂SO₄ at 40-80% saturation at 4°C. The precipitate fraction P₄₀₋₈₀ was applied to a CM-Sephadex C-50 ion-exchange column (2.5cm x 5.5cm), equilibrated and started with conditioning 0.5M citrate-phosphate buffer, pH 5.0, and followed by a NaCl gradient (0 - 1M). The flow rate was 27mL min⁻¹ and obtained the separation of only one fraction (F₅₀). The fraction F₅₀ was dialyzed, freeze dried and applied to a Sephadex G-100 gel filtration column (2.5 x 100.0 cm) equilibrated with a 0.1M citrate-phosphate buffer at pH 5.0. The elution flow was 17mL min⁻¹ with phosphate-citrate buffer (0.1M, pH 5.0) and resulted in one fraction F₁₀₀ with high specific activity (6.8UA mg⁻¹), indicating a possible enzyme separation. The electrophoresis in poliacrylamide gels of fraction F₁₀₀ stained with Schiff reagent, confirmed the presence of a separated band with glycoprotein nature.