

**Protocol for Electrocompetent *Lactobacillus casei* ATCC 334™ Cells Transformation**  
**Protocol code: CC\_Lc**

Materials:

- Laminar flow hood or sterile environment.
- 50mL and 500mL erlenmeyers.
- Micropipettes.
- Tips for micropipettes.
- 50 mL Falcon tubes.
- Refrigerate centrifuge for 50 mL Falcon tubes.
- 1.5 mL microcentrifuge tubes.
- Refrigerated microcentrifuge.
- 37°C incubator with and without shaking.
- *L.casei* ATCC 334™ culture.
- MRS media.
- MRS media supplemented (MRS + 1% de Glicina).
- Spectrophotometer (600nm).
- dH<sub>2</sub>O cold and sterile.
- PEG-8000 cold and sterile at 30%.

Procedure:

**Day 1:**

1. Set an overnight inoculum of *L.casei* ATCC 334™ in 15mL of MRS media at 37°C without shaking.

**Day 2:**

1. Set an inoculum of *L.casei* ATCC 334™ growth from last night in a preheated 200mL of MRS media supplemented with 1% glycine until a final OD<sub>600</sub> of 0.1.
2. Incubate at 37°C until get an OD<sub>600</sub> of 0.6.
3. Divide the final volume into 50mL Falcon tubes.
4. Centrifuge at 8500 rcf by 10 min at 4°C.
5. Discard the supernatant and resuspend the pellet in 1mL of dH<sub>2</sub>O cold and sterile, merge all tubes in only one and centrifuge again at 8500 rcf by 10 min at 4°C.
6. Discard the supernatant and resuspend the pellet in 3mL (final volume) of dH<sub>2</sub>O cold and sterile.
7. Aliquot 1mL in three 1.5mL microcentrifuge tubes and centrifuge them at 8500 rcf by 1 min at 4°C.
8. Discard the supernatant and wash the pellet twice with 1mL of dH<sub>2</sub>O cold and sterile and centrifuge at 8500 rcf by 1 min at 4°C.
9. Discard supernatant and resuspend in 1mL of sterile and cold PEG-8000 30% and centrifuge at 8500 rcf by 1 min at 4°C.
10. Discard supernatant and resuspend each pellet in 600µL of sterile and cold PEG-8000 30%.
11. Store at least overnight at -80°C and a maximum of two years at same temperature.