



INSTRUCTIONS TO PREPARE 1 LITER OF BOTUCRIO

*Keep the powder in the packets at -20°C until it's ready to be prepared.

1. **Warm up the OEP bottle in 40°C water bath and take 3mL. Important: After that proceeding you will get more than 3mL in the bottle, it is necessary to take only 3mL and throw the rest away.**

2. Add the **warmed 3mL** OEP to 850mL pure water (distilled or deionized)

3. SHAKE very well (spinning is not enough)

4. Add in 100mL egg yolk

5. SHAKE very well

6. Put in the content of the sachet (powder)

7. Add 0.5g of Gentamicine

8. SHAKE it until the mixture stays homogenous

9. Centrifuge at 3000rpm for 1 hour (Keep the mixture in the refrigerator while it's not been centrifugated)

10. Throw away carefully the precipitated and take the supernatant part.

11. Add 50mL of the cryoprotector to the supernatant obtained

12. Shake it very well.

13. Fractionate the amount into desired volumes

14. Store it at -20°C in the freezer

PS.: The frozen solution is valid to 24 months.