date:

23.10.2009

Operating Instructions

Electroporator Eppendorf 2510

Hazards for Humans and Environment

Risk of **liberation** of **genetically modified organisms (GMO)**
Risk of electrical shock

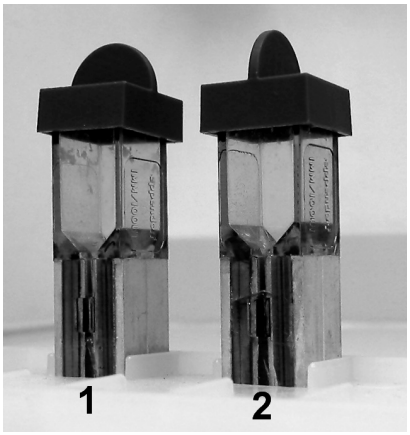
Protective Measures and Rules of Behaviour

Prepare electrocompetent cells (*i.e.* cells in a salt-free solution): Bacteria, yeast or cell lines
Electrocompetent bacteria or yeasts can be stored at - 20 °C (recommended - 80 °C)
All working steps should be done in an ice-bath:

- Resuspend the vector to be inserted into the cells in 5 µℓ A. dest. and transfer into a sterile Eppendorf tube
- Pre-cool the electroporation cuvettes: 10 mm gap for bacteria and yeast, 40 mm gap for cell lines
- Transfer sterile for each electroporation 1 mℓ of medium (LB, YC/Man-ol) into a sterile test tube
- Turn on electroporator, adjust voltage for the pulse: *E. coli* 1,250 V/10 mm, *Sc.c.* 750 V/10 mm
- Thawn electrocompetent micro-organism slowly on ice
- Add 40 µℓ cells to the 5 µℓ vector, mix softly by pipetting and transfer all the mixture into the electr. cuvette
- Transfer the electr. cuvette into the electroporator and pulse immediately by pressing twice "PULSE"
- Take off electr. cuvette and add immedeately 1 mℓ of medium (better fast than sterile)
  the medium is w/o selection pressure
- Transfer all cells into the Eppendorf tube and incubate at appropiate temp (37 °C or 30 °C) for 1 or 3 h:
  transfected cells start expressing the resistance gene introduced with the vector
- Plate 10 µℓ (vector) or 100 µℓ (ligation) of the cells onto selective agar plate
- Centrifuge the cells (30 s, 10,000 *g*) and plate remaining cells onto selective agar plates

Cleaning & Maintenance

	tap pos:
Cleaning electroporation cuvette for reuse:	
Add 1 mℓ 70 % EtOH to kill remaining GMO (overnight)	**1**
Add 1 mℓ 1 M HAc to delete residual vector DNA (4 h)	**1**
Wash thoroughly (10 x) with A. dest. to eliminate all acid	
Add 1 mℓ 70 % EtOH and store slightly taped until all EtOH has evaporated	**2**



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