## **Protocol for Cryopreservation of Muscle Tissue**

The aim of this protocol is to encourage all MH Investigation Units of the European MH Group to collect human muscle tissue of patients investigated by IVCT, in order to have the basis for primary human skeletal muscle cell cultures for future research projects.

## Material and methods:

## Important: all materials, solutions and muscle tissue must be handled absolutely sterile

- 1. DMSO/FCS/DMEM for cryopreservation:
  - 10 % DM50 (Merck)
  - 40 % FCS (Foetal Calf Serum) (Life Technologies)
  - 50 % DMEM (Dulbecco's Modified Eagle Medium),
    - 1000 mg glucose/l with sodium pyruvate
- 2. Nunc cryotubes (Nalge Company, P.O. Box 20365, Rochester, New York)
- 3. Nalgene Cryo 1°C Freezing Container, filled with isopropyl alcohol
- 4. Approximately 0.050 0.100 mg muscle tissue per cryotube (maximum length 5 mm and maximum diameter 5 mm)
- 5. Freeze **slowly** in the freezing container for 24 hours, then cryotubes must be transferred in liquid nitrogen
- Store in liquid nitrogen (alternatively at -70°C for a maximum time period of 8 - 12 weeks, long-term storage only in liquid nitrogen!)
- 7. Transport to another centre: only in dry ice, and as rapidly as possible (DHL etc.)

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