

Technical table for *Mentha*

Method 2: Vitrification with Cold Acclimation

| | Maintenance | Cold acclimation | Preculture | Loading | Dehydration | Rewarming | Recovery |
|--------------------------|---------------------------------------|---------------------------------------|---------------------------------------|--|--|---|--|
| Photoperiod | 16 h light/8 h dark | 8 h light/16 h dark | 8 h light/16 h dark | | | | 16 h light/8 h dark |
| Light intensity | 55 $\mu\text{M m}^{-2} \text{s}^{-2}$ | 27 $\mu\text{M m}^{-2} \text{s}^{-2}$ | 27 $\mu\text{M m}^{-2} \text{s}^{-2}$ | | | | Step 1: dark Step 2: 27 $\mu\text{M m}^{-2} \text{s}^{-2}$ Step 3: 55 $\mu\text{M m}^{-2} \text{s}^{-2}$ |
| Temperature | 25 \pm 3°C | 20°C light/-1°C dark | 20°C light/-1°C dark | 21°C | 21°C | Step 1: 45°C water bath Step 2: 25°C water bath Step 3: 21°C | 25 \pm 3°C |
| Transfer interval | 4-12 weeks | 1-6 weeks | 48 hours | 20 minutes | 20-30 minutes on ice | Step 1: 1 minute Step 2: 1-2 minutes Step 3: 20 minutes | Step 1: 48 hours Step 2: 72 hours Step 3: 4 weeks |
| Vessel used | Magenta® GA7* | Magenta® GA7* | Petri dish | Petri dish | cryovial | cryovial | Petri dish |
| Medium used | MS growth | MS growth | MS+5% DMSO | pre-chilled (0°C) MS+2 M glycerol+ 0.4 M sucrose | pre-chilled (0°C) 0.4 M sucrose+ MS PVS2 | Step 1: not applicable Step 2: not applicable Step 3: 1.2 M sucrose | Steps 1-3: BA+IBA mint recovery |