

Supplemental. 1

1. The preparation of basic medium (1000 ml)

| Ingredients | Quantity |
|--------------------------|--------------------------------------|
| DMEM | 5 g |
| F12 | 5.3 g |
| Streptomycin sulfate | 0.1 g |
| Hepes | 2.38 g |
| NaHCO ₃ | 2.388 g |
| DMSO(dimethyl sulfoxide) | 10 ml |
| Penicillin | 150 IU |
| 5.6% NaHCO ₃ | Adjust pH to 7.1-7.4 |
| Triple distilled water | Added into total volume with 1000 ml |

2. The preparation of cultural solution (50 ml)

12% BSA (bovine serum albumin) 5ml + basic medium 45ml

3. The preparation of vitrified solution

(1) Pre-equilibration solution: 1.5 mol/L (50 ml)

EG (ethylene glycol) 8.36 ml + 12% BSA 10 ml + basic medium 31.64 ml

(2) vitrified medium: 5.5 mol/L EG + 0.5 mol/L sucrose + 30% saccharosan (50 ml)

EG 15.3 ml + sucrose 8.56 g + saccharosan 15 g + 12% BSA 10 ml + basic medium 24.7 ml

4. Warming solution

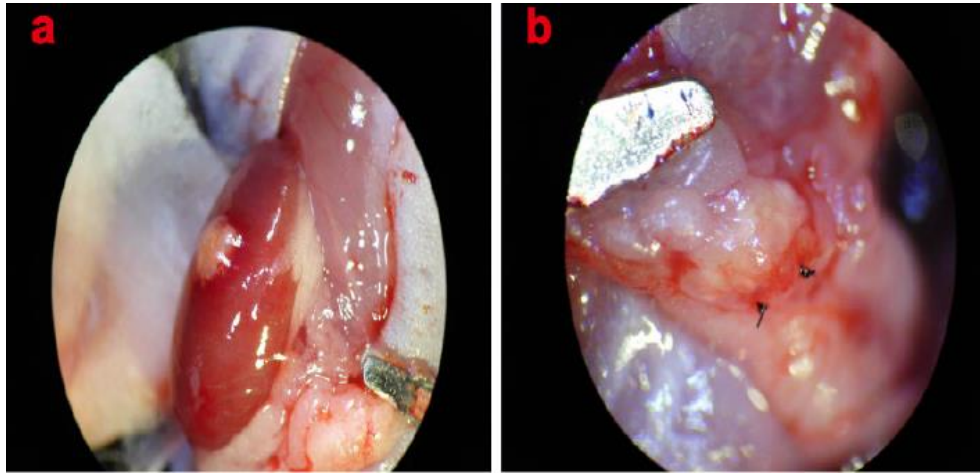
(1) 0.5mol/L Thawing solution (50ml): sucrose 8.56 g + 12% BSA 10 ml + basic medium 40 ml

(2) 0.25mol/L Thawing solution (50ml): 0.5mol/L Thawing solution 25ml + basic medium 25ml

(3) 0.125mol/L Thawing solution (50ml): 0.25mol/L Thawing solution 25ml + basic

medium 25ml

Supplemental. 2



Supplemental. 3

