

Mitochondrial Isolation from Skeletal Muscle

Reference: Isolation of Skeletal Muscle Mitochondria from Hamsters Using an Ionic Medium Containing Ethylenediaminetetraacetic Acid and Nagarse. Bhattacharya, S.K., *et al.*, Anal. Biochem., 192: 344-349, 1991.

| REAGENTS: | Sigma Catalog # |
|---|------------------------|
| Sucrose (MW=342.2) | S7903 |
| EDTA (MW=372.2) | E5134 |
| Tris-HCl (MW=157.6) | T3253 |
| KCL (MW=74.56) | P3911 |
| BSA (FA free) | A6003 |
| Mannitol (MW=182.17) | M9546 |
| K ₂ HPO ₄ (MW=174.18) | P3786 |
| Nagarse (~10U/mg) | 82518 |

BUFFERS:

| <u>Ionic Medium (IM):</u> | <u>for 100 mls</u> |
|---------------------------|--------------------|
| Sucrose (100 mM) | 3.422 |
| EDTA (10 mM) | 0.372 |
| Tris-HCl (100 mM) | 1.576 |
| KCl (46 mM) | 0.342 |
| pH 7.4 | |

IM +BSA:

| | |
|-------------------|-------|
| Sucrose (100 mM) | 3.422 |
| EDTA (10 mM) | 0.372 |
| Tris-HCl (100 mM) | 1.576 |
| KCl (46 mM) | 0.342 |
| BSA (0.5 %) | 0.5 |
| pH 7.4 | |

| <u>Suspension Medium (SM):</u> | <u>for 50mls</u> |
|--|------------------|
| Mannitol (230 mM) | 2.095 g |
| Sucrose (70 mM) | 1.198 g |
| EDTA (0.02 mM) | 0.0004 g |
| Tris-HCl (20 mM) | 0.1576 g |
| K ₂ HPO ₄ (5 mM) | 0.0435 g |
| pH 7.4 | |

Buffers can be prepared in advance and stored in fridge.

- Note that the protocol requires about 20 mls of IM and 30 mls of IM+BSA per sample processed.

PREPARATION:

- Fill 2 ice buckets.
- Label 2 60mm dishes for each sample. Place about 10 mls IM in one dish on ice.
- Prepare the following and keep on ice (make fresh on the day of prep):
 - 10 mg% Nagarse: 5.0 mg Nagarse + 50 mls IM
(prepare a little extra - 1 soln. for all samples)
 - 'Stop' tubes: Label a 50 ml conical tube for each sample, add 10 mls IM+BSA.
 - Oakridge tubes: Label one for each sample.

Note: Can go up to 20mg% Nagarse per the literature if needed

ISOLATION:

1. Harvest tissue and place into dish with cold IM on ice. Use entire hind limb musculature (both sides).
2. Trim away nerves, fat, and tendons and discard.
3. Place tissue on empty 2nd dish on ice (lid is best) and mince finely with fine scissors until it looks homogeneous.
4. Add 10 mls Nagarse solution and disperse with forceps into solution. Leave for 5 minutes on ice.
5. Pipet sample out of dish into labeled 50 ml conical tube containing 10 mls IM+BSA. Invert gently, allow tissue to settle and aspirate supernatant with 10ml pipet.
6. Re-suspend in 10 mls IM+BSA and transfer to homogenizer.
7. Homogenize (keeping homogenizer on ice and avoiding bubbles) with Eberbach device, about 5-7 passes (after Teflon pestle reaches bottom), until solution is uniform. Transfer back into 50 ml conical. Can leave on ice while preparing 2nd sample.

When both samples are ready, proceed to next step

8. Centrifuge suspension @ ~500g for 10 min at 4°C (2000 rpm in IEC centrifuge).
9. Transfer resulting supernatant to Oakridge tube and centrifuge @ 12,000g for 10 min at 4°C (12,000 rpm in Beckman JA-20 rotor).
10. Discard light colored fluffy upper layer (dislodge with gentle pipet action).
11. Resuspend the dark, tightly packed mitochondrial pellet in 10 ml IM+BSA (disperse with a small, pre-chilled glass tube).
12. Re-spin @ 12,000g for 10 min at 4°C, discard fluffy upper layer and supernatant.

13. Re-suspend final washed pellet in SM. Depending on pellet size, this requires about 200 μ l per animal. Gently remove pellet with a chilled glass test tube. Transfer to 1.5 ml tube on ice and pipet gently to suspend.
14. Quantitate protein. (1:20 and 1:50 dilutions of mitochondria usually works.)
15. Proceed to the respiration protocol
16. Use the same amount of protein per trace (typically 0.5mg)
17. After adding the 2mls of buffer C to the chamber, remove the amount of volume of mitochondria that you will add before adding the sample to the chamber.