Video Article

Mouse Adrenal Chromaffin Cell Isolation

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Abstract

Adrenal medullary chromaffin cell culture systems are extremely useful for the study of excitation-secretion coupling in an in vitro setting. This protocol illustrates the method used to dissect the adrenals and then isolate the medullary region by stripping away the adrenal cortex. The digestion of the medulla into single chromaffin cells is then demonstrated.

Video Link

The video component of this article can be found at https://www.jove.com/video/129/

Protocol

Abbreviations:
ACCs - Adrenal Chromaffin Cells, E.Soln - Enzyme Solution, E.DMEM - Enriched Dulbecco's Modified Eagle Medium

1. Preparation
    1. Add papain to desired quantity of E. Soln (40 units papain/ 1ml E. Soln.).
    2. Activate papain with carbogen for about 15 minutes on ice. Oxygenate Locke's buffer on ice as well.

2. Dissection
    1. Use 8-10 week mouse.
    2. Prior to digestion remove oxygenated Locke's buffer and place on ice.
    3. Refer to video for dissection procedure.
    4. Once excised, place glands in oxygenated Locke's buffer.

3. Preparation of Gland
    1. Remove fat from gland.
    2. Strip cortex from gland.

4. Enzymatic Digestion
    1. Sterile filter oxygenated papain.
    2. Make four pools of oxygenated papain on a petri dish.
        1. 3 pools should be about 100uls.
        2. 1 of the pools should be about 400uls.

    3. Wash medullae through pools
        1. 1st through the 3 100ul pools.
        2. Then through the 400ul pool
        3. Pippette 300ul E.Soln and medulla pieces from 400ul pool into microfuge tube.
        4. Qrap parafilm on microfuge tube and place in 37°C water bath for 20min.
        5. Remember to continue to oxygenate remaining E.Soln containing papain.
        6. After 20 minutes sterile filter more E.Soln.
        7. Replace old E.Soln bathing medullae with newly filtered E.Soln.

5. Triturations
    1. Aspirate and discard E.Soln.

2. Wash medullae in 1ml E.DMEM.
3. Aspirate and discard E.DMEM.
4. Add 300ul E.DMEM and triturate 20X with 1ml tip.
5. Cut 200ul tip with sterile razor blade.
6. Triturate 5-10X with cut 200ul tip.
7. Discard E.DMEM bathing medullae pieces. Medium will contain debris.
8. Add 300uls fresh E.DMEM to medullae pieces.
9. Triturate with 200ul tip 20x
10. Pippette E.DMEM to microfuge tube withough medullae pieces. Medium will contain free ACCs.
11. Add 300uls fresh E.DMEM to medullae pieces.
12. Triturate medullae with a 23.5 gauge syringe needle 20x.
13. Combine E.DMEM from both triturations. Medullae pieces should not be present and should now have a featherlike appearance.

6. Plating
    1. Microfuge E.DMEM from the last two trituration steps for 2.5 minutes at 3.7g s.
    2. Resuspend pellet in 50uls-200 E.DMEM, depending on subsequent experiments.
    3. Plate 10-30uls on a glass coverslip.
    4. Wait 15 minutes for cells to adhere.
    5. Add 750uls of E.DMEM.

Disclosures

The authors have nothing to disclose.

