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Single Cell Dissociation of Cultured Neurons

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We use this protocol and it's working

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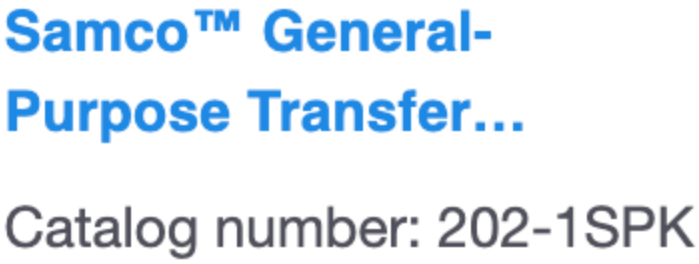
Abstract

Single-cell neuron dissociation is a crucial technique in cell culture that allows scientists to study individual neurons in isolation, providing insights into cellular mechanisms, gene expression, and neurodevelopmental processes. Here, we describe a method to obtain single neural cells from monolayer culture for use in MACS enrichment, FACS sorting, and re-culture.

Materials

Collagenase B 10x (4.0 U/mL). Sigma-Aldrich 11088807001
Collagenase P 10x (50.0 U/mL). Sigma-Aldrich l11213857001
Cell Disassociation Buffer Thermo Fisher Scientific 13151014
N2B27 Medium Gibco
Pen/Strep
CloneR2. StemCell Technologies 100-0691
BSA 30%, cell culture grade. Sigma cat # A9576
Benzonase Nuclease, Benzonase Nuclease HC, 90%, Size=25 ku
Thermo Scientific 202-1SPK with 7.9 mm wide tip



Thermo Scientific 202-1SPK

Material

Collagenase B 10x (4.0 U/mL). Sigma-Aldrich 11088807001
Collagenase P 10x (50.0 U/mL). Sigma-Aldrich I11213857001
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BSA 30%, cell culture grade. Sigma cat # A9576
Benzonase Nuclease, Benzonase Nuclease HC, 90%, Size=25 ku
Thermo Scientific 202-1SPK with 7.9 mm wide tip

Method

Preparation of Collagenase B Stock Solution (10x)

1. Collagenase B comes in a bottle containing 100 mg of lyophilized powder. The label indicates
   the amount of units/mg. (first bottle was 0.38 u/mg, i.e. ≈ 40 units).
2. Calculate the total amount of units in that bottle and hydrate its content with N2B27 Medium to
   make a stock solution of 4.0 units/ml.
3. Filter and aliquot 1 ml of the stock solution in 15 ml centrifuge tubes.
4. Store at -20 °C .
5. This solution is not sterile.

Preparation of Collagenase P Stock Solution (10x)

1. Collagenase P comes in a bottle containing 100mg of lyophilized powder. The label indicates the amount of units/mg.
2. Calculate the total amount of units in that bottle and hydrate its content with N2B27 Medium to make a stock solution of 50 units/ml.
3. Aliquot 500 ul of the stock solution in 1 ml appendorf tubes.
4. Store at -20 °C .
5. This solution is not sterile.

Preparation of Collagenase B (1x at 0.4U/ml) and P (1x at 5U/ml) Digestion Buffers:

1. Dilute at respective Collagenase stock solutions to 1x in N2B27 medium.
2. Prepare volume accordingly to get 3 mL per T25.

	A	B	C	D	E
	Reagents	stock []	working dilution	working []	vol (ul)
	Benzonase	25ku/ml	1:10000	0.0025	1.5
	BSA	30%	1:30	0.99%	100
	CloneR2	10x	1:10	1x	300
	Collagenase B	40units/ml	1:100	0.4unit/ml	30
	Penicillin-Streptomycin (10,000 U/mL)	100x	1:100	1	30
	1x N2B27				2307
	final volume (ul)				3000

Collagenase B Digestion Buffer.

	A	B	C	D	E
	Reagents	stock []	working dilution	working []	vol (ul)
	Benzonase	25ku/ml	1:10000	0.0025	1.5
	BSA	30%	1:30	0.99%	100
	CloneR2	10x	1:10	1x	300
	Collagenase P	50units/ml	1:10	5units/ml	300
	Penicillin-Streptomycin (10,000 U/mL)	100x	1:100	1	30
	1x N2B27				1767
	final volume (ul)				3000

Collagenase P Digestion Buffer.

5 **Prepare clean up medium**

1. Add 2 mL of 30% BSA to 2 mL of N2B27 to make 15% BSA. Keep these on ice until use.

6 **Prepare post sort medium**

1. Add 0.5 mL of BSA (30%) to 15 mL of N2B27 (final 1% BSA).
2. Add Clone R2.
3. Add GF specific for cell type of interest (NGF for neurons etc) as supplement to promote survival.

7 **Prepare sort tube**

1. Add 3 mL of sort medium per 15 mL falcon tube.
2. Roll the tube horizontally to coat the entire side wall (this very important to prevent loss of cells to the side of the tube.

Cell Disassociation (GENTLE IS THE RULE)

2h 37m

8 2h 43m

1. Remove culture medium
2. Add freshly made Collagenase B Digestion Buffer, 3 mL/ T25.
3. Incubate 01:00:00 at 37 °C in the incubator .
4. Add freshly made Collagenase P Digestion Buffer to Collagenase B Digestion Buffer/Cell suspension, 3 mL/ T25.
5. Incubate 01:00:00 at 37 °C in the incubator .
6. Collect cell suspension in 15 mL tube with large caliber transfer pipette (7.9 mm).
7. Centrifuge 00:05:00 at 400g.
8. Add 1 mL of Cell Dissociation buffer.
9. Suspend cell pellet by flicking the tube..
10. Incubate 00:10:00 at 37 °C water bath .
11. Add 4 mL of **ice cold** sort medium (N2B27 + 1 % BSA + 1x Clone R2).
12. Incubate on On ice ice for 00:15:00 .
13. Triturate gently by aspirating up and down with a large caliber transfer pipette.
14. Pipette up and down slowly with a 1 mL pipette.
15. Centrifuge 00:05:00 at 400g pre-cooled to 4 °C .
16. Suspend by flicking the tube in 1 mL of sorting medium and reserve on ice.

> Note
>
> Axon stumps and disrupted cells are removed by adding **ice cold** 15% BSA gradient centrifugation.

1. Add 3 mL of ice cold **clean up medium** to a new 15 tube.
2. Gently layer the cell suspension on top of the **clean up medium.**
3. Centrifuge 00:08:00 at 120g at 4 °C .

4. Remove the supernatant carefully and keep aside.

Note

Axon stumps and disrupted cells are mostly floating in the supernatant. Viable cells are in the pellet.

1. Check supernatant for viable cells and recovery.
2. Add 2 mL of sort medium to the pellet. Re-suspend pellet by flicking the tube. DO NOT PIPETTE
3. Pass cell through cell strainer (70 µM).
4. Perform cell counts and adjust concentration accordingly for sorting.
- Ideally for good sort low cell density at 1 million per ml maximum.

Protocol references

PKA-RII subunit phosphorylation precedes activation by cAMP and regulates activity termination.
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