Miltenyi Biotec

Neurosphere Dissociation Kit (P)

Order no. 130-095-943

Contents

1. Description

This product is for research use only.

Components	**Neurosphere Dissociation Kit (P)** 6 vials, containing: 2.5 mL of Enzyme P 2×50 mL of Buffer X (sterile) 1.5 mL of Buffer Y (sterile) 1 vial (5–15 mg*) of Enzyme A 1 mL of Buffer A
Size	For 100 digestions of 1 mL.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. Reconstitute Enzyme A before the date indicated on the vial label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1.

*The vials contain a consistent amount of units. Variability in ranges of mg within the vial is possible – according to variability of activity per mg

1.1 Principle of the Neurosphere Dissociation Kit (P)

The Neurosphere Dissociation Kit (P) provides a simple and fast procedure to enzymatically dissociate mouse, rat, or human neurospheres into single cells.

After harvesting, the neurospheres are digested enzymatically and further mechanically dissociated into a single-cell suspension by trituration.

1.2 Background information

The Neurosphere Dissociation Kit (P) has been designed for a gentle but rapid and efficient generation of single-cell suspensions from cultivated neurospheres. Neural stem cells isolated from whole mouse brain proliferate under special culture conditions forming clonally derived spheres, called neurospheres. Dissociated cells can be quantified and frequently subcultured to generate more neurospheres, as well as analyzed *in vitro* for phenotype distributions or other functional studies.

1.3 Applications

- Dissociation of neurospheres to single-cell suspensions for subsequent cultivation and expansion of neurospheres.
- Phenotyping, functional, and genetic studies of neural stem cells by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- Hanks´ Balanced Salt Solution (HBSS) with Ca^{2+} and Mg^{2+} (Sigma-Aldrich # 55037C), in the following referred to as HBSS (w)
- 2 mL, 15 mL, and 50 mL tubes
- Pre-Separation Filters (30 µm) (# 130-041-407)
- (Sterile) glass Pasteur pipettes
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubation oven at 37 °C

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2. Protocol

2.1 Reagent and instrument preparation

▲ Volumes given below are for the dissociation of 40 mL of neurosphere suspension. When working with less than 40 mL, use the same volumes as indicated, When working with more than 40 mL, scale up all reagent volumes and total volumes accordingly.

1. Resuspend the lyophilized powder in the vial labeled Enzyme A with 1 mL of Buffer A. Do **not** vortex. This solution should then be aliquoted and stored at –20 °C for later use.
2. Fire-polish three glass Pasteur pipettes so that decreasing tip diameters are achieved. For details refer to 3. Appendix.

2.2 Neurospheres dissociation protocol

▲ All steps should be performed under sterile conditions.

▲ The MACSmix Tube Rotator is used with slow, continuous rotation.

1. Pre-heat 960 µL of Buffer X for up to 40 mL of neurosphere suspension at 37 °C for 10–15 minutes before use.
2. Detach neurospheres by tapping the flask against the benchtop and pass a stream of media across the attached neurospheres. Harvest suspended neurospheres and transfer the suspension to an appropriate-sized sterile tube (50 mL).
3. Centrifuge at 300×g for 10 minutes at room temperature and aspirate the supernatant carefully.
4. Resuspend the cell pellet in 960 µLof pre-heated Buffer X.
5. Add 25 µL of Enzyme P, 10 µL of Buffer Y, and 5 µL of Enzyme A to the neurospehere suspension and mix gently.
6. Transfer the neurosphere suspension to a 2 mL tube.
7. Incubate the closed tube for 10 minutes at 37 °C under slow, continuous rotation using the MACSmix™ Tube Rotator.
8. Dissociate neurospheres mechanically using the wide-tipped, fire-polished Pasteur pipette by pipetting up and down 10 times slowly. Avoid forming air bubbles.

   ▲ **Note:** To remove cells stuck to the tube wall, rinse the tube wall once or twice with the sample solution.
9. Incubate at 37 °C for 5 minutes using the MACSmix Tube Rotator.
10. Dissociate neurospheres mechanically using the other two fire-polished Pasteur pipettes of decreasing diameter. Pipette slowly up and down 10 times with each pipette, being careful to avoid the formation of air bubbles.
11. Apply the single-cell suspension to a Pre-Separation Filter (30 µm) placed on a 15 mL tube.
12. Apply 5 mL of HBSS (w) through the Pre-Separation Filter (30 µm).
13. Discard filter and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate the supernatant completely.
14. Cells should be processed immediately for further applications or resuspend in fresh media for subsequent cultivation after determination of the cell number.

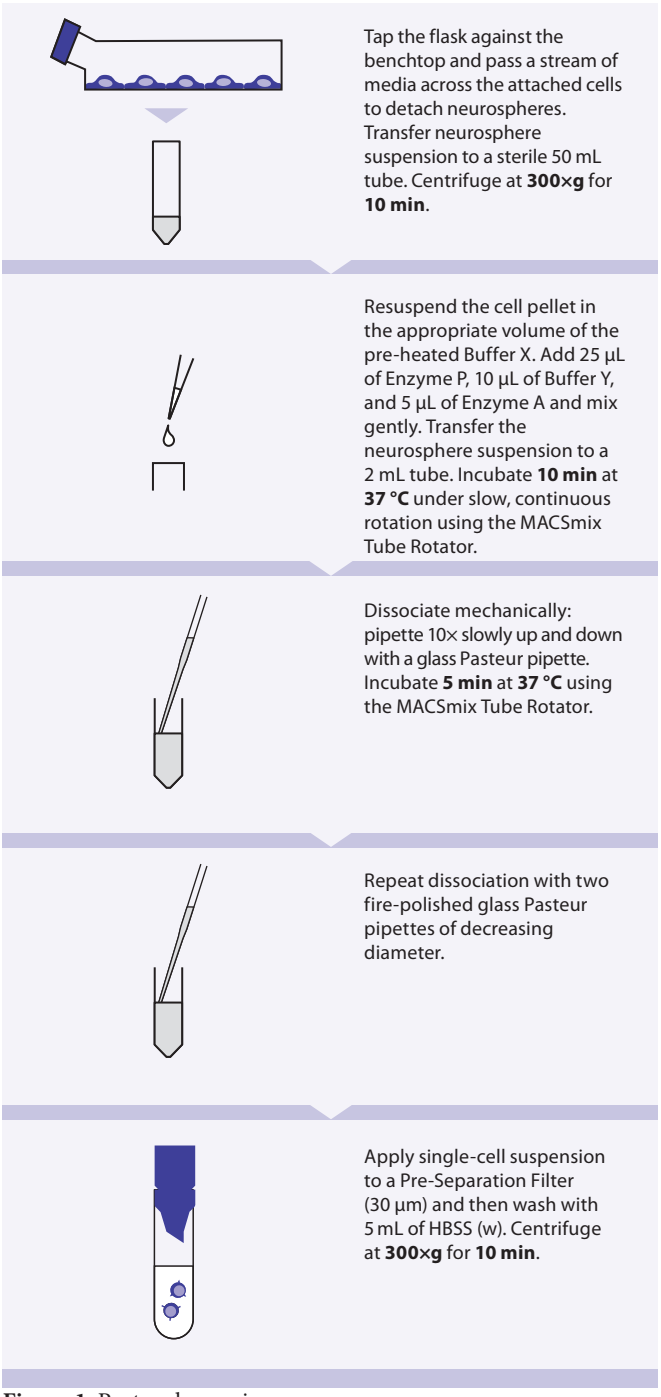

Figure 1: Protocol overview

3. Appendix: Tips & hints

▲ For up-to-date information regarding antigen compatibilities of the Neurosphere Dissociation Kit (P) for subsequent MACS® Cell Separations, please refer to www.miltenyibiotec.com/130-095-943.

Production of appropriate Pasteur pipettes
For this protocol three Pasteur pipettes with openings of decreasing diameter are needed. The opening of the first pipette should be rounded without significant decrease in the size of the opening. The smallest opening should not be smaller than 0.5 mm so that the cells are not forced through with too much pressure. To produce openings that get progressively smaller, rotate the pipettes quickly in the flame to fire polish them for a few seconds. Production is easier if you apply the rubber sucker. Too much time may fuse the hole. The edges should be rounded.

Yield of viable cells is too low (dissociation is insufficient)
Make sure that the neurospheres are agitated sufficiently during the entire time of incubation and do not stick to the bottom of the tube. Flick the tube after adding the enzyme mixe if it is necessary. During the working steps at 37 °C the MACSmix™ Tube Rotator is convenient for this purpose. Cell clumps will not get through the Pre-Separation Filter. Therefore, keep pipetting until the single-cell suspension looks homogeneous. Then apply suspension to the Pre-Separation Filter.

Single-cell suspension contains only dead cells
Make sure the openings of the Pasteur pipettes are not too small. Pipette more slowly and do not vortex the cells. Avoid forming bubbles. Follow the protocol non-stop.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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