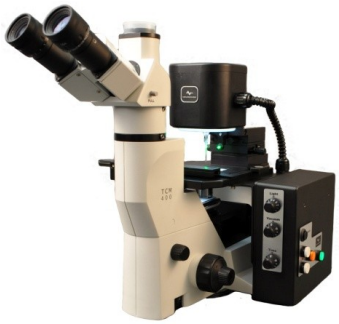
Microdissection of native brain tissue with Kuiqpick™ for neural progenitor cultures: An optimized protocol



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Introduction

Kuiqpick™ is an efficient and effective way for the isolation of subanatomical regions from live brain tissue slices. The vacuum pulls the cells into the capillary with minimal mechanical damage, and large areas can be collected quickly using disposable capillaries (DCU) with greater inner diameters. This protocol describes the technique established to isolate living cells from live brain sections for primary neural progenitor cell culture. A simple protocol for the collection of various subanatomical brain regions and the successful subsequent recultivation of stem cell cultures is described below†.

Materials

- Vibratome
- Thermostated bath
- Kuiqpick™
- Surgical instruments
- Artificial Cerebro Spinal Fluid (ACSF) - 300-500ml containing (in mM): 126 NaCl, 1.25 $NaH2PO_4$, 26 $NaHCO_3$, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, and 10 D-glucose, pH 7.4, carbogen (95% O_2 5% CO_2). May be replaced by tissue culture medium balanced to carbogen.
- Dissociation solution: 1mM EDTA, 0.05% Trypsin
- Hanks Balanced Salt Solution (HBSS) Invitrogen?
- Neurobasal media with supplements and growth factors: Neurobasal media containing 1% (v/v) Penicillin/ Streptomycin; 2μM (final concentration) L-glutamine; 0.04% (v/v) Heparin, 2% (v/v) B-27; 20ng/ml fibroblast growth factor, FGF2; and 10ng/ml epidermal growth factor, EGF.
- Ice

Specimen preparation may be performed according to standard protocols. Described below is the preparation used for Kuiqpick™ protocol.

Tissue preparation

1. ACSF may be prepared a night before and cooled to 4°C in a refrigerator.
2. 10-20 minute before experiment aerate ACSF with carbogen.
3. Anesthetize the animal with 50 mg/kg Nembutal or other anesthetic.
4. Open the chest and perfuse the animal through the heart aorta with cold aerated ACSF for 3-5 minutes.
5. Excise the brain, rinse with cold ACSF, remove excess liquid with sterile napkin and glue the brain on the vibratome platform with a fast glue (e.g. medical device adhesive Loctide 4014).
6. Slice 200-300μm sections and place them in aerated ACSF on ice.

Microdissection with Kuiqpick™

Optimization of parameters (vacuum strength and duration) may be required depending on the type of brain subanatomical regions microdissected and tissue preparation.

7. Place a tissue slice on the slide surface and cover it with aerated ACSF using a dropper or a pipettor.
8. Select a disposable capillary unit (DCU) with desired internal diameter (ID) and calibrate it on the slide adjacent to the slice as described in the Calibration section of Kuiqpick™ manual. *(For adult tissue DCUs with ID 100μm diameter and greater are recommended.)*
9. Set both dials for vacuum strength and duration to 4. Identify the target area and position it precisely under the DCU tip using the microscope crosshair.
10. Initiate dissection of the area of interest by pressing Sample button. Move to the next adjacent area and continue collection until the desired region is removed. If necessary adjust the vacuum duration and strength for optimal collection.
11. When the DCU shaft is filled, or when the desired amount of sample has been collected, remove the DCU and position the tip above or just inside a microcentrifuge tube on ice. Attach a loaded syringe to the luer of the DCU and slowly release the microdissected sample into the microcentrifuge tube.

Endnotes

† Protocol and data published in PloS One, 2012. Kudo LC, Vi N, Ma Z, Fields T, Avliyakulov NK, Haykinson MJ, Bragin A, and Karsten SL (2012) Novel Cell and Tissue Acquisition System (CTAS): Microdissection of Live and Frozen Brain Tissues. PloS One. 7(7):e41564. Epub 2012 Jul 24.

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