NEUROINDX

KuiqpicK™ 1.2

Cell and Tissue Acquisition System

User Manual

Contents

1 Introduction

KuiqpicK™ 1.2, a capillary-based cell and tissue acquisition system, is the key to reliable, precise, and affordable cell and tissue sample acquisition prerequisite for a range of *in vitro* studies, including cell and region specific tissue experimentation and single cell analysis.

This vacuum-assisted capillary based system coupled to an inverted microscope allows for rapid and efficient acquisition of specific cells from adherent cell cultures based on their morphology, location or fluorescent label. It can also collect single cells from suspension cell cultures and individual multicellular spheres from three-dimensional (3D) cell cultures. **KuiqpicK™** collects cells without compromising cell viability, thus enabling primary culturing or recultivation of the collected single cells.

In addition to the collection of individual cells from culture dishes, KuiqpicK performs isolation of single cells and microdissection of subanatomical regions from various brain tissue samples prepared by different methods, such as fresh frozen tissue, sucrose treated tissue and fresh live tissues, with minimal contamination of surrounding components, while leaving the intracellular structure and molecules intact. Moreover, it works with thicker tissue sections up to 500 µm, suitable for an analysis that requires large amounts of sample material, such as proteomics.

Samples collected using **KuiqpicK™** may be used for a wide range of downstream applications and techniques used in modern molecular biology, targeting both protein and nucleic acids, including, but not limited to, quantitative RT-PCR, global gene expression, epigenetic, and proteomics studies.

2 Safety

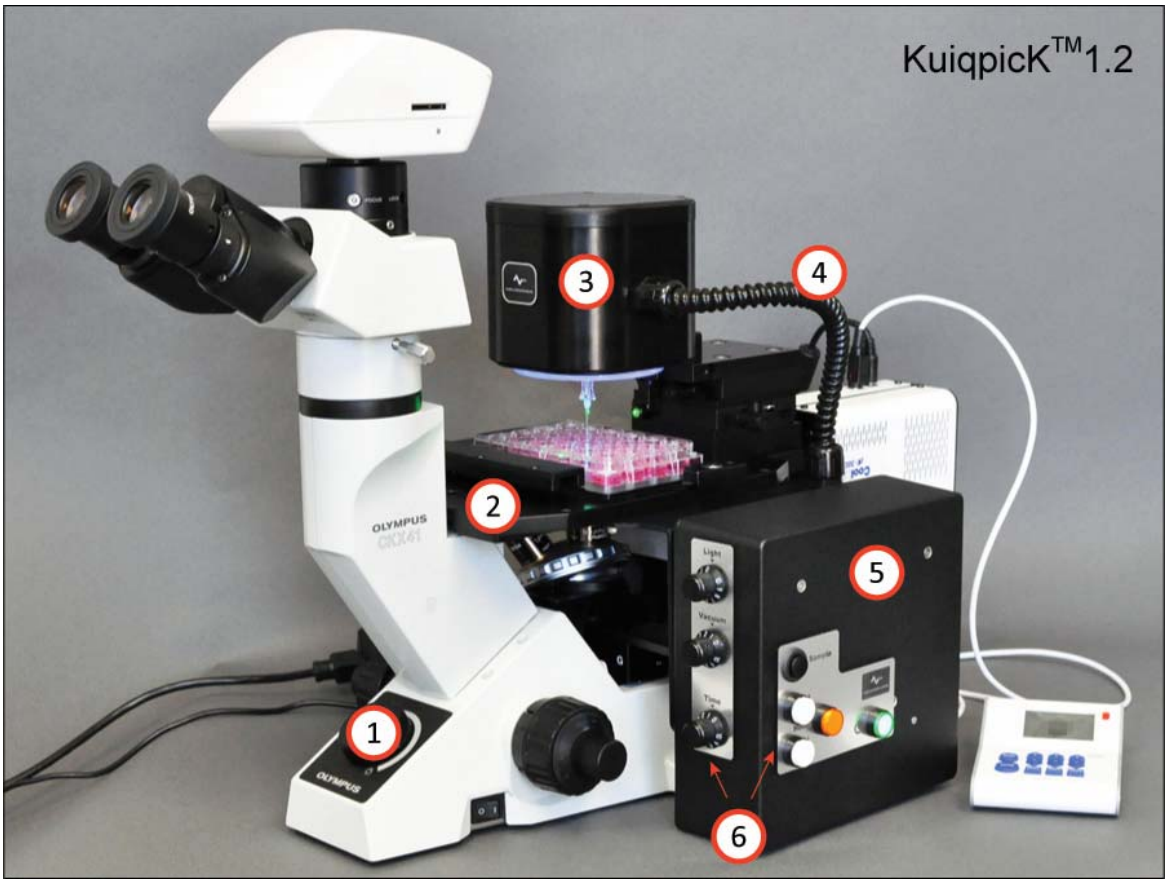
1. Handle glass capillaries with care. Capillaries have sharp points and can break on or under skin surface if accidently contacted; therefore, it is strongly recommended to always wear goggles and gloves during handling.
2. Install **KuiqpicK™** microscope on a sturdy, level surface and allow at least 5 inches of space all around **KuiqpicK™** microscope for ventilation.
3. Always use the power supply and cord provided by with the unit. If a proper power supply and cord are not used, product safety performance cannot be warranted.
4. When installing **KuiqpicK™**, route the power cord away from the microscope frame and **KuiqpicK™** unit.
5. Always ensure that the grounding terminal of the **KuiqpicK™** unit and that of the wall outlet are properly connected. If the unit is not grounded, NeuroInDx cannot warrant electrical safety.
6. Never allow metal objects to penetrate any openings on the microscope frame or **KuiqpicK™** unit as this could result in user injury and damage to the instrument.
7. Do not insert objects into the capillary holder other than filters and disposable capillary units (DCUs).
8. When **KuiqpicK™** is not in operation, be sure to turn the power off and disconnect the power cord from the connector socket of the **KuiqpicK™** unit or from the wall power outlet.
9. Dispose used capillaries (DCUs) into a biohazard glass waste container according to regulations.

Caution: KuiqpicK™ components contain hazardous materials. **KuiqpicK™** should be disposed according to local regulations.

3 Components and Accessories

KuiqpicK™ 1.2 comes equipped with an Olympus CKX41 inverted microscope (Olympus America Inc.), **KuiqpicK™** unit (Sampler Head at top with adjustable LED ring light, Vacuum Line and Electric Cables, Control Box with vacuum module), and a power supply with cord. Ready-to-use Disposable Capillary Units (DCUs) attachments for cell collection and brain tissue dissection and filter attachments are sold separately.

NOTE: **The camera, camera adapter and fluorescence illumination system as shown in the figure below are not included and must be purchased separately.**



1. Inverted microscope with mechanical stage
2. XY stage mount
3. **KuiqpicK™** Sampler Head
4. **KuiqpicK™** Vacuum Line and Electric Cables
5. **KuiqpicK™** Control Box
6. Light adjustment dial, vacuum dials and DCU controls

4 Initial Set Up

All necessary components are included in the packing. Make sure that the following items have been received upon opening the package.

1. **KuiqpicK™** and microscope (Olympus CKX41) body
2. Mechanical XY stage (x1)
3. Trinocular observation head (x1)
4. Eyepiece (x2)
5. Crosshair reticle with reticle holder (x1)
6. Objectives (one of each: 4X, 10X, 20X)
7. Hex wrench (x1)
8. Stage plate (round silver)
9. Stage plates (black set of 3)
10. Power supply and cord for **KuiqpicK™** (one of each)

Proper installation of all parts is required for **KuiqpicK™** to work properly. Make sure the following steps are completed before attempting to use the system. Refer to the Olympus CKX41 manual for operation and installation specific to the microscope.

Objectives

Lower the revolving nosepiece using the coarse adjustment knob towards the back. Remove the dust prevention cap.

Screw the objectives starting with the lowest magnification into the revolving nosepiece from the left side of the microscope. Turn the nosepiece clockwise and mount the remaining objectives in ascending magnification order.

Objectives should be cleaned periodically.

Cover unused threaded position with the dust prevention cap to prevent foreign objects from entering.

Observation Head

1. Loosen the head locking screw on the observation head and remove the dust cover caps on the dovetail cavity and the observation head.

2. Mount the observation head by engaging the dovetail at the bottom of the head into the cavity in the microscope's arm.
3. Tighten the head locking screw after positioning the observation head as desired.

Eyepieces

1. Remove the protective caps from the observation tubes.
2. Insert the reticle in one of the 10x eyepieces and secure it by screwing in the reticle holder.
3. Insert the 10x eyepieces into the ocular sleeves.

Interpupillary Distance (IPD) and Diopter

1. Looking through both eyepieces, move the left diopter until both left and right fields of view perfectly coincide. This is your proper IPD.
2. Looking through the right eyepiece only, use the coarse and fine focus adjustment knobs to bring the specimen into focus. Adjust the setting to "0" on the left eyepiece and look through with the left eye. Bring the specimen into focus using only the diopter setting.

Power supply and cord

Connect the 24V power supply and the cord. Plug the female cord plug into the power jack located in the rear inside wall of the **KuiqpicK™** side chassis and plug the power cable into the outlet.

Optional Accessories

KuiqpicK™ is equipped with a trinocular port. A camera and/or video system can be mounted with an appropriate adapter (not supplied). Contact your local Olympus sales representative for appropriate adapter and camera options.

NOTE: **The power switch and the light intensity adjustment knob on the Olympus CKX41 microscope are nonfunctional on a KuiqpicK™ 1.2 unit.**

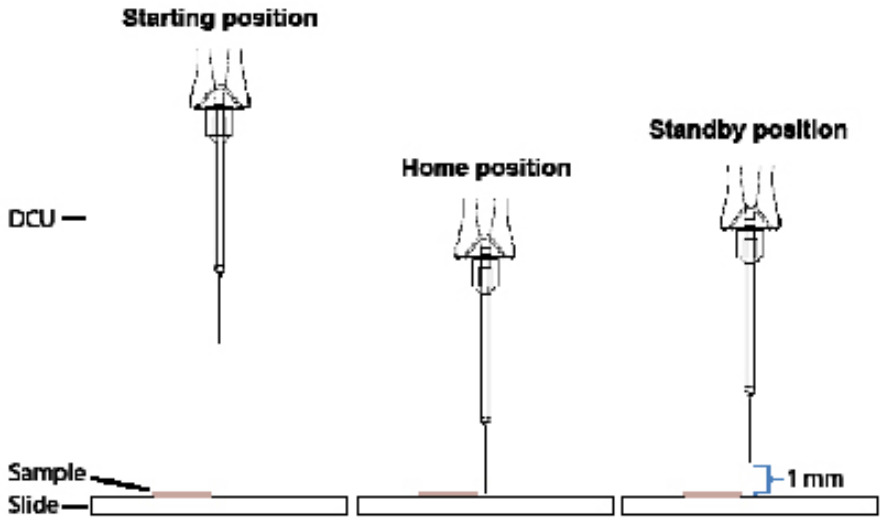
5 Using KuiqpicK™

Terminology

Starting position: Highest vertical position for the DCU; the initial position when the unit is first turned on and capillary has been mounted or after it is reset.

Home position: Calibration of Collecting/Dissecting position of the DCU tip determined by the user. Homing is performed prior to every collection/dissection procedure.

Standby position: 1 mm above the Home position. DCU will be lifted to Standby position when the **Orange** Home button is pressed (**Figure 1**). DCU returns to Standby position after each sampling until the Home position or the system has been reset.



Power

To turn power on, press the **Green** pushbutton on the side chassis. The button will illuminate in depressed position when turned on. Press again to turn unit off (**Figure 1**).

Filter and DCU attachment

Lift the **KuiqpicK™** head back to expose the male luer connector. Holding the outer rim of the filter, connect the female luer lock connector of the filter to the male luer connector on the **KuiqpicK™** head. Next, attach a DCU to the filter. As the **KuiqpicK™** head is brought back down, the green horizontal LED will light (**Figure 2**).

Caution: DCU consists of two parts, the hub base with a female luer lock connector and a glass capillary component. Always handle a DCU by the hub base to avoid injury and damage to the capillary.

X-Y control

To center the tip of the DCU in the field of view, use the manual dials (**Figure 2**) on the linear stages at the base of the **KuiqpicK™** head where it mounts to the microscope stage.

Positioning

To position the tip of the DCU along the z-axis, use the **White** buttons (**Figure 1**) to move up and down. A single quick press will move the DCU by 1.5 µm. When either of the **White** buttons is held down for more than 5 seconds, the DCU will move at a rate of 350 µm/s.

Home

Use the **White** *Positioning* buttons to set the Home position for sampling, i.e. the desired position of the DCU's tip on the z-axis for sampling (**Figure 1**). When the capillary tip comes in contact with the tissue surface, press the **Orange** Homing button (**Figure 1**). When the Home position is designated, the Home button will illuminate and DCU will move 1 mm above that position on the z-axis to the Standby position.

Vacuum level and duration control

The two dials at the front of the side chassis (**Figure 1**) control the vacuum level from 2''Hg (1) to maximum of 22''Hg (10) and the vacuum duration from 100 ms (1) to maximum of 1 s (10). Depending on the sample and the desired result, various vacuum level and duration may be used. We recommend testing these parameters prior to any collection/dissection.

Sample

After the Home position has been set, pressing the **Black** "Sample" button (**Figure 1**) will initiate sample collection by bringing the DCU down to the Home position and activating the vacuum at the selected strength and duration.

Figure 1: ***KuiqpicK™*** *1.2 controls include: (1) Power on/off (**Green** button); (2) Positioning (**White** buttons): to bring the capillary up or down towards the stage; (3) Home (**Orange** button): to calibrate capillary (DCU) for cell and tissue collection and set it in standby position; (4) Light intensity (top dial); (5) Vacuum (middle dial): increasing vacuum pressure from 1 (minimum, 2 inches of mercury) to 10 (maximum, 22 inches of mercury); (6) Duration (bottom dial): increasing vacuum duration in increments of 100 milliseconds, from 1 (shortest, 100 milliseconds) to 10 (longest, 1 second); (7) Sample (**Black** button): for cell/tissue collection.*

Figure 2: ***KuiqpicK™*** *head (1) in its half-lifted position for disposable capillary (DCU) attachment/removal, shown here with filter (2) and DCU (3) attached. In the lifted position, the green horizontal LED light (7) is automatically turned off. It is automatically turned on when the* ***KuiqpicK™*** *head is in its upright position. The ring LED lights (4) stay on in a fully lifted position. The x-y position of the DCU is controlled by the knobs (5) on the linear stages (6). The knobs on the right and left sides of the bottom stage control the side to side movement, and those on the front and back of the stage control the front to back movement of the DCU to center it above the mechanical XY stage.*

a. Initial KuiqpicK™ Training Exercises

For instructional videos visit our website: www.neuroindx.com or YouTube channel: http://www.youtube.com/NDXInc

1. Start **KuiqpicK™** by pressing the **Green** power button once (the button will light up when depressed). Lift Head to attach filter and DCU as described in "Calibration" section.

Caution: Wear gloves and protective eyewear when handling DCUs containing glass capillaries.

2. Place a marking on a histological glass slide and place it on the mechanical stage with 2 drops of distilled water.
3. Using the **White** Positioning buttons, carefully bring the DCU down until the capillary tip touches the surface of the liquid.

NOTE: **Quick button pressing will move the capillary 1.5 µm per step. Holding down the button for 5 seconds will increase the speed to 350 µm/s**

4. When in focus, the annulus of the capillary tip appears as a bright green ring.
5. Use the knobs on the linear stages (**Figure 2**) to locate the green annulus ring.
6. Locate the marking on slide. Using coarse and fine focus adjustment knobs to focus on marking.
7. Carefully bring the DCU down until the annulus of the capillary tip comes into focus as a bright green ring.
8. Stop lowering the DCU when the capillary tip moves with the glass slide when using the mechanical stage. It is helpful to move the slide/plate using the mechanical stage to determine if the DCU tip has made contact with the slide/plate surface. When the DCU tip is in contact with the surface, a slight motion of the mechanical stage will result in a movement of the capillary tip.
9. Lift the Head to reset **KuiqpicK™**.
10. Bring the head back down and repeat steps 1 through 7.

b. DCU Calibration for Tissue Microdissection

Each DCU must be calibrated before sampling. For instructional video visit our website: www.neuroindx.com or our YouTube channel: http://www.youtube.com/NDXInc

NOTE: **Depending on the region of interest, DCUs of different internal diameters (IDs) should be used. The size of desired cells and cell clusters shall determine the appropriate diameters of the DCUs. An average interneuron is about 15-25µm. For microdissection of brain anatomical regions (e.g. mouse hippocampus) ~50µm ID DCU is recommended. Subanatomical regions, such as dentate gyrus or cortical layers, may be collected with ~30µm ID DCUs. If fine microdissection is required, DCUs with <30µm diameters should be used.**

Wear gloves and protective eyewear when handling DCUs containing glass capillaries.

1. Start **KuiqpicK™** by pressing the **Green** power button once (the button will light up when depressed)
2. Lift the **KuiqpicK™** head (**Figure 2**) to attach filter and DCU.

Caution: DCUs have sharp tips! Handle with care. Improper handling may cause injuries. Never contact the capillary tip to avoid injury and contamination.

3. To attach the filter and DCU, lift the head, and attach a filter directly to the male luer connecter on the **KuiqpicK™** sampler head.
4. Carefully handle new DCU by its hub base and attach directly onto the filter.

Caution: Always use a filter with the DCU to prevent biological material and liquids from entering KuiqpicK's mechanical parts. When KuiqpicK™ is not in use, attach a new filter unit to prevent foreign objects from entering the system until next use.

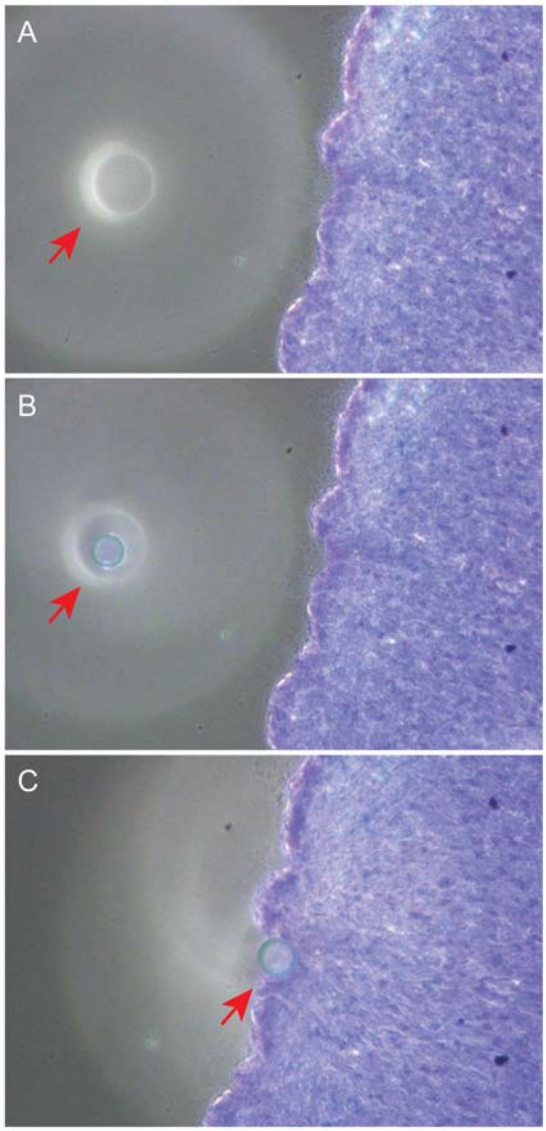
5. Place a tissue slide on the stage and add buffer to the tissue. Bring **KuiqpicK™** head back down.
6. Position the slide to a clear spot on the slide adjacent to the tissue sample for calibration.
7. Using the **White** *Positioning* buttons, carefully bring the DCU down until the capillary tip touches the surface of the buffer.

NOTE: **Quick button pressing will move the capillary 1.5 µm per step. Holding down the button for 5 seconds will increase the speed to 350µm/s. Use caution.**

8. Use the **linear stages** (micromanipulators) to locate the DCU halo and position the halo adjacent to the tissue (**Figure 3A**).

9. Use coarse and fine focusing knobs to focus on the tissue. Slowly bring the DCU down until the annulus of the capillary tip comes into focus as a bright green ring (**Figure 3B**). Lower the DCU until the capillary tip comes in contact with the tissue (**Figure 3C**). It is helpful to move the microscope slide with the mechanical stage in order to determine if the DCU tip has made contact. If the DCU tip has made contact with the tissue proceed to step 11. If the DCU tip has made contact with the slide surface, a slight motion of the mechanical stage will result in the simultaneous movement of the capillary tip.

10. If the tug of the slide movement is observed, press the **White** *Positioning* up button several times until the motion of the mechanical stage no longer pulls the capillary tip. This assures that the lowest travel of the capillary tip will not break the tip upon downward movement during dissection.



11. Using the **linear stages** align the annulus of the capillary tip to the center of the ocular crosshairs**.**

Caution: Bringing down the DCU too low will cause the capillary tip to break against the sample slide or plate. If you suspect that a tip has been broken, be sure to check the surrounding area for broken glass before replacing the DCU to avoid injury or damage.

Figure 3: *Three step procedure for DCU calibration.* ***A:*** *Initial finding of DCU tip (halo, shown with red arrow) in the field of vision;* ***B:*** *Lowering the DCU until the DCU tip and tissue section are both in focus;* ***C:*** *Moving the XY mechanical stage to test if the DCU tip is in contact with the tissue section surface.*

12. Press the **Orange** Home button (the button will light up when calibrated) to set the calibrated Home position. The DCU will remain at the Standby position 1 mm above the Home position, i.e. sampling position.

13. To re-calibrate, simply press both **White** buttons simultaneously to bring the DCU back to Home position.

Re-position the capillary tip using the **White** Positioning buttons and then push the **Orange** button to set the new Home position. To cancel re-calibration, press both **White** buttons simultaneously instead of pressing the Home button. This will bring the DCU back up to the previous Standby position.

14. You may re-calibrate at any time during the sampling procedure to designate a new Home position.
15. Proceed to **Cell/Tissue Collection Section**

NOTE: **When the KuiqpicK™ Head is lifted, the green horizontal light will turn off and the DCU will reset by moving up to its initial starting position. The DCU must then go through the calibration process again.**

Caution: When the KuiqpicK™ head is lifted, the DCU will return to its initial starting position. Do not try to detach the DCU immediately after lifting the KuiqpicK™ head. Wait until the DCU stops moving completely to avoid causing damage to the KuiqpicK™ sampler unit.

c. DCU Calibration for Adherent Cell Cultures

NOTE: **Each DCU must be calibrated before sampling. For instructional video visit our website: www.neuroindx.com or YouTube channel: http://www.youtube.com/user/NDXInc**

Caution: Wear gloves and protective eyewear when handling DCUs containing glass capillaries.

TIP: When working with attached cells it is recommended to wash cells with fresh medium to remove dead cells.

1. Start **KuiqpicK™** by pressing the **Green** power button once (the button will light up when depressed)
2. Lift the **KuiqpicK™** head (**Figure 2**) to attach filter and DCU.

Caution: DCUs have sharp tips! Handle with care. Improper handling may cause injuries. Never contact the capillary tip to avoid injury and contamination.

3. Attach filter directly to the male luer connecter on the **KuiqpicK™** head.
4. Carefully handle a new DCU by its hub base and attach it directly onto the filter.

Caution: Always use a filter with the DCU. The filter will prevent biological material and liquids from entering KuiqpicK's mechanical parts. When KuiqpicK™ is not in use, attach a new filter unit to prevent foreign objects from entering until next use.

5. Place a cell plate/dish on the stage and lower the **KuiqpicK™** head.
6. Using the **White** *Positioning* buttons, carefully bring the DCU down until the tip of capillary touches the surface of the liquid. Quick button pressing will move the capillary 1.5µm per step. Holding down the button for 5 seconds will increase the speed to 350µm/s. **Use caution.**
7. Use the linear stages (**Figure 2**) to locate the DCU halo.
8. Use coarse and fine focusing knobs to focus on the cells. Slowly bring the DCU down until the annulus of the capillary tip comes into focus as a green ring. Position the green ring over a clear spot. Continue to lower the DCU until the tip has made contact with a cell. It is helpful to move the plate/dish with the mechanical stage in order to determine if the DCU tip has made contact with a cell or the plate/dish surface. If the DCU tip is just above the slide surface, the edge of the cell will press against the capillary tip (See figure below). If contact is made, proceed to step 10.
9. If the DCU tip is in contact with the slide surface, a slight motion of the mechanical stage will result in the simultaneous movement of the capillary tip. If the tug of the slide movement is observed, press the up button several times until the motion of the mechanical stage no longer pulls the capillary tip. This assures that the lowest travel of the capillary tip will not break the tip upon downward movement during dissection.
10. Using the linear stages align the annulus to the center of the ocular crosshairs (**Figure 2**).

Caution: Bringing down the DCU too low will cause the capillary tip to break against the sample slide or plate. If you suspect that the tip has been broken, be sure to check the surrounding area for broken glass before replacing the DCU to avoid injury or damage.

11. Press the **Orange** Home button (the button will light up when calibrated) to set the calibrated Home position. The DCU will remain on Standby position 1 mm above the sampling position.
12. To re-calibrate, simply press both **White** buttons simultaneously to bring the DCU back to Home position. Re-position the capillary tip using the **White** Positioning buttons and then push the **Orange** button to set the new Home position. To cancel re-calibration, press both **White** buttons simultaneously instead of pressing the Home button. This will bring the DCU back up to the previous Standby position.

13. You may re-calibrate at any time during the sampling procedure to designate a new Home position.
14. Proceed to **Cell/Tissue Collection Section**

NOTE: **When the KuiqpicK™ head is lifted, the green horizontal light will turn off and the DCU will reset and move up to its initial starting position. The DCU must then go through the calibration process again.**

Caution: When the KuiqpicK™ head is lifted, the DCU will return to its initial starting position. Do not try to detach the DCU immediately after lifting the KuiqpicK™ head. Wait until the DCU stops moving completely to avoid causing damage to the KuiqpicK™ sampler unit.

d. Cell/Tissue Collection

1. Adjust values for the white LED ring light, vacuum pressure, and duration.

NOTE: **Optimal vacuum levels and duration can vary depending on the type of cell culture, cell confluence, and tissue type. It is recommended that a test sample be used to determine the optimal values of vacuum level and duration prior to sampling. Start at the lowest setting for vacuum level and duration and slowly increase them in turn.**

2. Position the area for acquisition on the cell plate/tissue slide to the center of the ocular crosshairs where the DCU has already been positioned. Under the microscope, the annulus of the capillary tip is where the cell/tissue will be collected
3. Push the **Black** Sample button to acquire the targeted cell/tissue
4. Working quickly, repeat until desired number of cells/tissue has been collected or until DCU has reached its capacity. **Stop when the capillary shaft is filled just below the hub with buffer.**
5. Make sure that a syringe with a male luer lock has been prepared with the plunger slightly pulled back and a filter attached to the tip (**Figure 4**).To empty the contents from the DCU, lift the **KuiqpicK™** head and carefully detach the DCU. Affix the DCU to the filter on the prepared syringe (**Figure 4**). Using both hands hold the DCU in a receptacle (e.g. microcentrifuge tube) and eject contents by gently pushing the plunger down.

Caution: The fine tip can be fragile when it comes in contact with the tube wall. Handle the DCU extremely carefully to avoid to break the DCU tip when transfer the collected samples. Moreover, because small amounts of capillary contents can

spill during syringe attachment, the tip should be held within a tube while attaching the syringe.

6. Attach DCU to **KuiqpicK™** head and re-calibrate to continue collection.

e. DCU Re-Calibration Function after Lifting KuiqpicK™ Head

After setting the Home position for sampling, collecting your samples, and lifting the head to remove your DCU the instrument will remember your Standby position.

***NOTE:* Because of the slight variability in the lengths of the DCUs avoid the use of this feature when switching DCUs to prevent accidental breakage of the DCU.**

1. Attach the same DCU to **KuiqpicK™** and bring the head back down to its upright position.
2. Press both **White** Positioning buttons simultaneously to bring the DCU down to the previous Standby position.
3. Calibrate by lowering the DCU until the capillary tip reaches the same level as your tissue/cells.
4. Press the **Orange** Home button to set the new Home position, which will lift the DCU by 1mm to the new Standby position.
5. Press the sample button to collect tissue/cell.

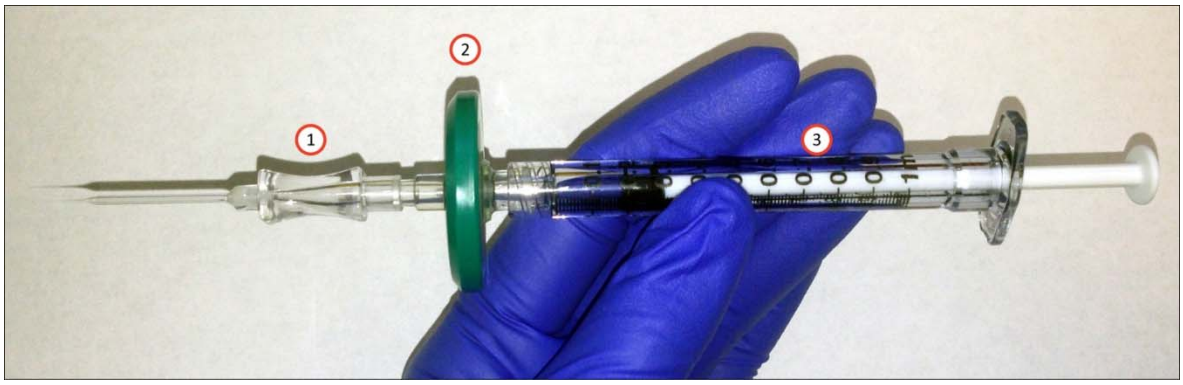


Figure 4: *DCU and filter affixed to a luer lock sterile syringe. 1 – DCU; 2 – filter, 3 – syringe.*

6 Sample Protocols

a. Protocol 1: Collection of Individual Adherent Cells from Culture Dishes

Various adherent cell cultures including human neuroblastoma cell line SH-SY5Y, Chinese hamster ovary (CHO) cells, human melanoma MDA-MB-435 cells and various primary cultures, such as neural progenitors, skin fibroblasts, etc., can be used for the collection of individual cells using **KuiqpicK™**. The collected cells are viable and may be used for recultivation and clonal expansion, as well as for single cell analysis, including protein and nucleic acid analyses. See Application Notes on our website: http://www.neuroindx.com/KuiqpicK/application-notes/

NOTE: **Recommended medium for neuroblastoma cell line SH-SY5Y, Chinese hamster ovary (CHO) cells and human melanoma MDA-MB-435 cells (ATCC, Manassas, VA): Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) (Life Technologies, Grand Island, NY) supplemented with 10% (v/v) heat-inactivated fetal calf serum containing 2mM glutamine and 1% antibiotics (penicillin–streptomycin). Cells should be maintained at 37°C in a humidified 5% CO_2 atmosphere. For some tightly adherent cells, we recommend non-enzymatic Cell Dissociation Solution (e.g. Sigma; C5789)**

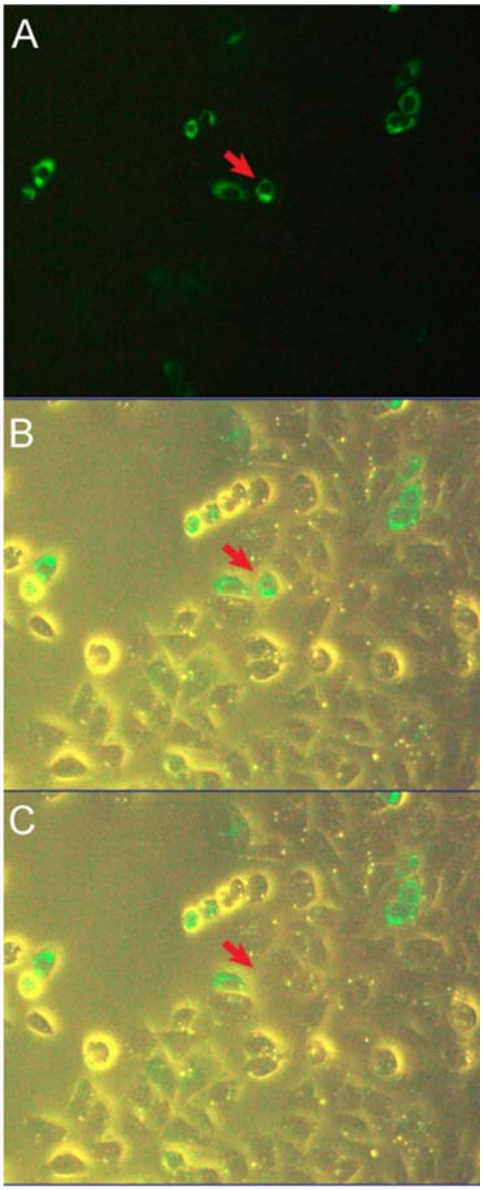
Cell collection with KuiqpicK™ from cultures: It is recommended for cell cultures to not exceed 50% confluence for single cell collection, although collections may be performed at higher confluence. Prior to cell collection, media should be removed from the plate/dish and cells should be gently washed once with fresh pre-warmed medium to remove dead floating cells. Replenish media and place plate/dish on microscope stage. Proceed with cell collection as described in **Calibration for Cell Cultures** and **Cell Collection** sections.

Selecting DCU Size: DCU size should be selected based on the size of cells of interest and the confluence of cultures. DCUs with internal diameter (ID) greater than the cells are recommended when collecting cells for recultivation and clonal expansion, i.e. when collecting cells of 20µm diameter, a DCU with ID 30µm should be used to maximally maintain cell viability. DCUs with <30µm diameters is recommended when working with smaller cells or cultures with >70% confluence. However, DCUs with smaller ID may cause some damage to cells.

Collecting Individual Cells: To collect cells, position the desired cell under the center of crosshairs and press the **Black Sample** button. DCU is lowered and cell is aspirated into the capillary. It is recommended that no more than 30 minutes be spent per plate. However, timeframe for cell collection is dependent on cell type and sensitivity.

When collecting fluorescently labeled cells (**Figure 5**), calibrate the DCU under bright field as described above, then turn off LED ring light Turn on the fluorescent illumination to locate

desired labeled cells. Turn the LED ring light on and position the cell under the center of the crosshair. Turn fluorescent illumination off to prevent bleaching of cells. After collecting the cell, fluorescent illumination may be turned on again and the process is repeated.



NOTE: **Optimal vacuum levels and duration can vary depending on the type of cell cultures and confluence of the cells. It is recommended that a test cell culture dish be used to determine the optimal values of vacuum level and duration prior to sampling. Start at the lowest settings for vacuum level and duration and slowly increase them in turn.**

Ejecting Cells From DCU: Prior to removing a DCU from **KuiqpicK™** head, pull the syringe plunger back and attach a filter to the syringe tip (**Figure 4**). Carefully remove the DCU from **KuiqpicK™** head and affix to the filter on syringe. Carefully position the DCU tip into preloaded media or buffer and slowly eject cells.

Reculturing and Clonal Expansion Preconditions: Some cell types require preconditioned media (same media where the cells were grown) in order to grow single cell or a small number of cells. If cells do not take after attempting to reculture, eject collected cells into filtered preconditioned media in which the cells were originally cultured. The culture conditions to grow single cells would be optimized by users, which will vary depending on cell types.

Figure 5: *Collecting fluorescently labeled cells.* ***A:*** *Initial identification of fluorescently labeled cells under fluorescent illumination.* ***B:*** *Under bright field fluorescent label is still visible. Bright field is used to position the cell of interest under the crosshair for collection and to visualize cell collection into the DCU. Fluorescent illumination should be turned off to prevent bleaching of label prior to sampling.* ***C:*** *Upon collecting the cell, turn on fluorescent illumination to resume collection.*

NOTE: **Change the DCU and filter after each collection procedure. DCUs may be reused by washing with a syringe and sterilizing with ethanol. DCUs can also be autoclaved. However, NeuroInDx recommends using a new DCU for each collection procedure to avoid contamination.**

b. Protocol 2: Sucrose Treated Frozen Brain Tissue Microdissection

This protocol describes the isolation of single cells, cell clusters, and subanatomical regions from sucrose treated brain slices. Sucrose treated brain tissue keeps brain morphology intact, and thus ideal for microdissection. Furthermore, this optimized protocol yields high quality RNA from the microdissected material.

Materials:

- Surgical instruments
- Standard animal perfusion apparatus and setup
- Cryostat
- Standard Phosphate Buffered Saline (PBS)
- Sucrose (15-20%) in PBS filtered
- 2-methylbutane
- Dry ice
- Glass microscope slides
- 0.025% Cresyl violet, 0.01% toluidine blue, hematoxylin, or any vital dyes
- Pipettor and sterile pipette tips
- **KuiqpicK™** disposable capillary units (DCU) with appropriate internal diameter (ID) for the application. ID≤30 µm single cell collection. ID≈20-100 µm for subanatomical regions.

Recommended tissue preparation:

Flush animal with standard preperfusion PBS (phosphate buffer saline). Remove the brain and sink in 15-20% Sucrose in PBS at 4°C overnight. Flash freeze the brain in 2-methylbutane on dry ice. If not for immediate use, store tissue at -80°C. Prepare cryosections from 10 to 100 µm thickness. For single cell collection with **KuiqpicK™**, cut sections at ≤30 µm. Stain slides with 0.01% toluidine blue or 0.025% cresyl violet for 10 seconds. Wash with standard PBS. Also, see Application Notes on our website: http://www.neuroindx.com/KuiqpicK/application-notes/

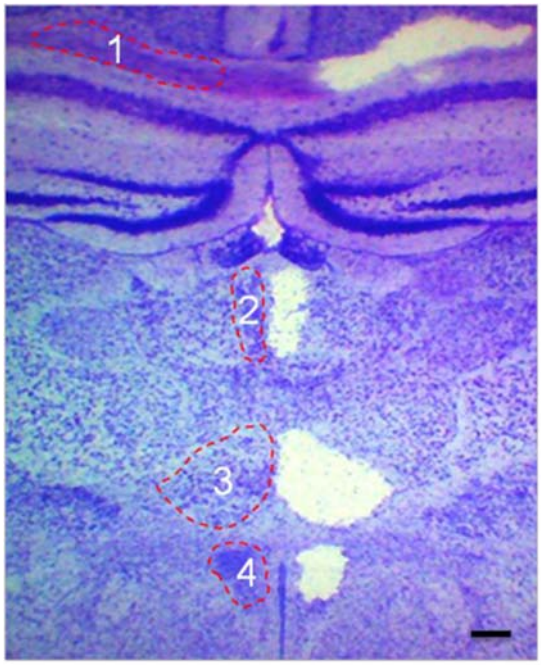
Caution: Dyes should be used at lower than standard concentrations. Overstaining will cause stiffening of tissue samples, thus making them difficult to microdissect.

Microdissection or Collection of Single Cell from Tissue Slices:

Dry the back of the slide after PBS wash and place the slide on **KuiqpicK™** stage. It is critical to keep the tissue section moist at all times by adding either PBS or 15% sucrose in PBS during longer sessions of microdissection. High quality RNA may be isolated from brain tissue samples

collected within 1 hour. Attach a DCU to **KuiqpicK™** head. Follow instructions in **DCU Calibration for Tissue Microdissection** and **Cell/Tissue Collection** sections.

NOTE: **Optimal vacuum levels and duration can vary depending on the type of samples being dissected. It is recommended that a test slide be used to determine the optimal values of vacuum level and duration prior to sampling. Start at the lowest settings for vacuum level and duration and slowly increase them in turn.**



Selecting DCU Size: DCUs of different diameters should be used based on the size of the cells and subanatomical regions of interest. ID≤30µm for single cell collection and ID≈20-100µm for subanatomical regions is recommended.

Figure 6. *Unilateral microdissection of the corpus callosum (1), paratenial thalamic nucleus (2), nucleus reunions (3) and hypothalamic nucleus (4). Tissue was stained with toluidine blue. Tissue thickness 50 µm. Scale bar= 250µm*

NOTE: **Check the DCU carefully during dissection to make sure that the samples are being collected into the capillary. If not, repeat calibration or adjust vacuum level and duration.**

Caution: Stop when the capillary shaft is filled to <u>just below the hub</u> with buffer. Detach DCU and transfer collected samples as described above to avoid liquid transfer beyond the filter into the system.

Ejecting Cell/Tissue from DCU:

Prior to removing a DCU from **KuiqpicK™** head, pull the syringe plunger back and attach a filter to the syringe tip. Carefully remove the DCU from **KuiqpicK™** head and affix to the filter on syringe. Carefully position the DCU tip into preloaded buffer and slowly eject cell/tissue. Cells/tissue may be ejected into a microcentrifuge tube containing the desired buffer for subsequent application. To remove remaining tissue inside the capillary shaft, carefully and slowly load the attached syringe with PBS to rinse the DCU and then release the contents into the microcentrifuge tube. If not for immediate use, samples may be frozen on dry ice and then stored at -80°C.

NOTE: **Change the DCU and filter after each collection procedure. DCUs may be reused by washing with a syringe and sterilizing with ethanol. DCUs can also be autoclaved. However, NeuroInDx recommends using a new DCU for each collection procedure to avoid contamination.**

c. Protocol 3: Microdissection of Native Brain Tissues

KuiqpicK™ is able to dissect live tissues for primary culture and various downstream molecular analyses. The following is a sample protocol for the collection of live cells from brain tissue to be used for culturing. **KuiqpicK™** can be applied to dissect live cells from other tissues, such as tumors, for primary culture using suitable protocols. Under appropriate extracellular and sterile conditions ensuring cell viability, fresh adult brain tissue containing live cells may be used for dissection for up to 8 hour. [1] See Application Notes on our website: http://www.neuroindx.com/KuiqpicK/application-notes/

Materials:

- Vibratome
- Thermostat bath
- Surgical instruments
- Artificial Cerebrospinal Fluid (ACSF) 300-500ml containing (in mM): 126 NaCl, 1.25 NaH_2PO_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). ACSF may be replaced by tissue culture medium balanced to carbogen.
- Dissociation solution: 1mM EDTA, 0.05% Trypsin
- Hanks Balanced Salt Solution (HBSS)
- Neurobasal media with supplements and growth factors: Neurobasal media (NB+++, see below) 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

NOTE: **ACSF may be prepared the night before and cooled to 4°C in a refrigerator.**

Tissue Preparation: Specimen preparation may be performed according to standard protocols. Described below is the preparation used for KuiqpicK™ protocol.

[1] Practical Electrophysiological Methods: A Guide for In Vitro Studies in Vertebrate Neurobiology. (1992) Eds. Helmut Kettenmann and Rosemarie Grantyn, Wiley-Liss, New York

1. Aerate ACSF with carbogen 10-20 minute before the experiment.
2. Anesthetize the animal with 50 mg/kg Nembutal or other anesthetic approved by your ARC.
3. Open the chest and flush the animal through the aorta with cold aerated ACSF for 3-5 minutes.
4. Excise the brain, rinse with cold ACSF, and remove excess liquid with sterile napkin.
5. Keep brain in buffer consisting of Krebs-Ringer solution saturated with carbogen (95% O_2, 5% CO_2) on ice until mounted onto glass slides.
6. Glue the brain on the vibratome platform with fast glue (e.g. medical device adhesive Loctide 4014).
7. Slice 200-300µm sections and carefully place a tissue slice onto a glass slide and keep moist with ACSF. Keep slides on ice.

Tissue Microdissection and Collection:

8. Dry the back of the slide and place it on KuiqpicK™ stage. Cover the slide surface with aerated ACSF using a dropper or a pipettor and follow instructions in **DCU Calibration for Tissue Microdissection** and **Cell/Tissue Collection** sections.

Caution: Stop when the capillary shaft is filled to <u>just below the hub</u> with buffer. Detach DCU and transfer collected samples as described above to avoid liquid transfer beyond the filter into the system.

***NOTE:* DCUs with ID 100µm diameter and greater are recommended for adult rat brains.**

***NOTE:* For sample dissection video visit our website: www.neuroindx.com or YouTube channel: http://www.youtube.com/user/NDXInc**

Ejecting Cell/Tissue from DCU:

9. Prior to removing a DCU from **KuiqpicK™** head, pull the syringe plunger back and attach a filter to the syringe tip.
10. Carefully remove the DCU from **KuiqpicK™** head and affix to the filter on syringe.
11. Carefully position the DCU tip into preloaded buffer and slowly eject tissue into a 1.5mL tube.
12. Add 50 µL of 0.05% trypsin.
13. Incubate at 37°C for 10 minutes.

14. Add 450 µl of Hank's balanced saline solution (HBSS) and centrifuge at 1500 rpm for 5 minutes.
15. Remove the supernatant.
16. Add 800 µl of NB+++ and dissociate cells by aspirating with a pipette.
17. Plate the cells and incubate at 37°C in a 5% CO_2 atmosphere.

Media

NB+++ (50 ml)

Neurobasal medium	48 ml
Penicillin/Streptomycin (100X)	500 µl
L-glutamine (200 mM), [2 mM final]	500 µl
Heparin (5 mg/ml), [2 µg/mL final]	20 µl
B-27 (50X)	1 ml
FGF2 (25 ng/µl), [20 ng/ml final]	40 µl
EGF (100 ng/µl), [10 ng/ml final]	5 µl

d. Protocol 4: Microdissection of Microcapillary Cell Walls from Mouse Heart Muscle Tissue

This protocol is suitable for dissecting most difficult–to-dissect tissue and isolating high quality RNA from collected samples.

Materials:

- Liberase DL Research Grade (Roche, REF 05-401-160-001), 5mg vial
- Proteinase inhibitor (Sigma tablets; SigmaFast S8820)
- Kolliphor® P188 (Sigma 15759) also known as Pluronic F-68
- 1M sterile $CaCl_2$
- MEM without L-glutamine, FBS, or antibiotics (Corning)
- 1x Phosphate buffer saline (PBS)
- Cryostat
- Ice

Reagents:

Prepare prior to the day of microdissection:

1. **P188 stock solution** (15mM; 100x stock solution), place in a 37˚C water bath to dissolve, filter sterilize and store at 4˚C in the dark
2. **Liberase DL Stock Solution**
    a. Slowly thaw enzyme (Liberase DL, lyophilized form) on ice for 30 min
    b. Add 2ml of MEM, alternatively 1x PBS may also be used
    c. Keep on ice, resuspend by pipetting. Do not vortex. Stock may be kept at 4°C for up to a month. Alternatively, aliquot into smaller volumes (200 µl) and store at -20°C. Use or discard an aliquot after thawing. Do not re-freeze

Prepare on the day of dissection

1. **Dissociation Solution**, mix the following components to make 10ml of solution
    a. 200µl Liberaze stock solution
    b. 75µl 1M $CaCl_2$
    c. 100µl P188
    d. MEM to 10ml (MEM may be replaced by 1xPBS)
    e. Place on ice
2. **Inhibitor Solution**
    a. Add 1 proteinase inhibitor tablet in 250ml MEM or 1xPBS

Tissue Preparation:

1. Anesthetize the animal with 50 mg/kg Nembutal or other anesthetic approved by your ARC.
2. Flush or perfuse the animal with standard PBS.
3. Remove the heart and other organs and sink them in 15-20% Sucrose in PBS at 4°C overnight.
4. Flash freeze heart and organs in 2-methylbutane on dry ice. If not for immediate use, store tissue at -80°C.
5. Prepare cryosections from 10 to 25µm thickness.
6. Stain slides with 0.025% cresyl violet for 10 seconds and gently wash with PBS.
7. Apply Dissociation Solution to sections and incubate at room temperature for no more than 30 minutes.
8. Tilt the slide onto an absorbent surface (e.g. paper towel) and soak away the dissociation solution.

9. Gently wash tissue sections with Inhibitor Solution.

Tissue Microdissection and Collection:

10. Dry the back of the slide and place it on KuiqpicK™ microscope stage.
11. Cover the slide surface with Inhibitor Solution using a dropper or a pipettor and follow instructions in **DCU Calibration for Tissue Microdissection** and **Cell/Tissue Collection** sections.

Caution: Stop when the capillary shaft is filled to just below the hub with buffer. Detach DCU and transfer collected samples as described above to avoid liquid transfer beyond the filter into the system.

NOTE: **Selecting DCU Size: DCUs with ID≤30µm for single cell collection, ID≈20-100µm for subanatomical regions, and ID≈60-80µm for microcapillaries are recommended.**

NOTE: **For sample dissection video visit our website: www.neuroindx.com or YouTube channel: http://www.youtube.com/user/NDXInc**

Ejecting Cell/Tissue from DCU:

12. Prior to removing a DCU from **KuiqpicK™** head, pull the syringe plunger back and attach a filter to the syringe tip.
13. Carefully remove the DCU from **KuiqpicK™** head and affix to the filter on syringe.
14. Carefully place the DCU tip into a microcentrifuge tube preloaded with buffer and slowly eject cell/tissue. If there is remaining tissue inside the capillary shaft, carefully and slowly load the attached syringe with Inhibitor Solution to rinse the DCU and then release the contents into the microcentrifuge tube.
15. Spin cells at 500 x g for 5 minutes and remove the supernatant to remove the Inhibitor Solution.
16. Samples may then be used immediately or frozen on dry ice and stored at -80°C for later use.

References

1. Alisina, Janivette, Leach Steven D., and Baily Jennifer M. (2013). The incorporation of polaxamers during pancreatic tissue dissociation allows isolation of high quality RNA from FACS-sorted pancreatic cells. The Pancreapedia: Exocrine Pancreas Knowledge Base, DOI:10.3998/panc.2013.10

7 Troubleshooting

If problems with **KuiqpicK™** occur by factors other than manufacturing defects, please review the following guide. If you encounter any other problems, please contact technical support.

Problem	Cause	Solution
1. Tissue or cell is not being picked up into the DCU.	Vacuum level and/or duration are/is not high/long enough.	Increase vacuum pressure and/or duration.
		Use a DCU with larger tip diameter.
	DCU tip is clogged.	Maintain humidity between tissue and the DCU tip.
		Change to a new DCU.
	DCU tip is not touching the sample slide.	Re-calibrate the DCU.
	DCU tip has been damaged.	Change to a new DCU.
2. Too much background light for capturing microscope images.	Green horizontal LED light on **KuiqpicK™** head.	Block off light source with masking tape or other opaque object.
	Reflection of light off of the DCU's glass capillary shaft.	Bring DCU up with **White** Up button.
3. DCU does not move by pushing the **White** Positioning buttons.	DCU is too short and has reached its lowest position.	Restart **KuiqpicK™** by powering off and on.
		DCU may have been broken. Change to a new DCU.
4. Too much liquid is accumulated in the capillary shaft or filter gets clogged.	Vacuum level and/or duration are set too high.	Decrease vacuum level and/or duration. Monitor the capillary shaft during collection. Stop collection when it is filled to just below the hub with buffer.
		Change the filter attachment.
5. Bubbles appear on the surface of tissue slide.	The tissue section has been fixed before sectioning.	Make sure that the tissue is not fixed.
	Tissue sections are thick.	Mount tissue sections with thickness of 50 µm or less.

6. **Orange** Home button does not light up.	**White** *Positioning* buttons were simultaneously pressed repeatedly or Homing button was pressed without bringing down the DCU at least 1mm.	Reset DCU to starting position by lifting up **KuiqpicK™** head or powering off/on.
7. **Orange** Home button blinks continuously.	There is a leak in the vacuum line.	Make sure that the blue tubing behind the control box is secure.
	The movement of the DCU in the sampler unit is obstructed.	Check to see if there is anything obstructing the movement of the DCU and remove it.

8 Technical Specifications

Specification	Description	
1. Illumination	Light source	144 LEDs ring light
	Input	24VDC
2. Focusing mechanism	Stage height adjustment mechanism Fine adjustment scale: 0.002 per graduation; Fine adjustment stroke: 0.2mm per turn Total stroke: 28mm Co-axial coarse and fine focusing on ball drive Coarse adjustment travel per rotation	
3. Observation tube	Field number: 22 Tube tilting angle: 30° Interpupillary distance adjustment range: 52-75	
4. Stage	Size	240 x 160 mm (with mechanical X-Y stage)
	Movement range	114 x 41.3mm
	Specimen holder	Slide and Petri dish
5. Dimensions & Weight	20.0 in/508mm (L) x [11.5 in/292 mm (min W)~ 15 in/381mm (max W)] x 16 in/406.5mm (H)	
6. Pump	Vacuum range	up to 22”Hg
	Input	24VDC
7. Linear actuator	Linear travel/step	0.0015 mm
	Maximum travel	8.9 mm
	Input	5 VDC
8. Operating environment	Indoor use Altitude: max. 2000 meters Ambient temperature: 5°C to 40°C (41°F to 104°F) Maximum relative humidity: 80% for temperature up to 31°C (88°F) Supply voltage: 100VAC to 240 V AC, 50-60 Hz	

9 System Performance

Description	Specifications
Resolution	Single Cell
Vacuum duration (Ts), seconds	0.1 s to 1.0 s
Vacuum strength, Hg"	4.4" to 22" Hg
Available DCU IDs, µm	From 10 to100 µm
Acquisition speed (Hg"/Ts), seconds	
Minimum settings (4.4"/0.1 sec) Maximum settings (22.0"/1.0 sec)	1.3 s 2.2 s
Acquisition sample volume (Hg"/Ts/DCU ID) *	
4.4"/0.1 sec/20 µm 22.0"/1.0 sec/20 µm 4.4"/0.1 sec/30 µm 22.0"/1.0 sec/30 µm 4.4"/0.1 sec/40 µm 22.0"/1.0 sec/40 µm	10 to 30 nl 1.4 to 2.2 µl 35 to 55 nl 1.5 to 2.8 µl 70 to 100 nl 4.7 to 4.9 µl
Cell collection speed (cells/minute) from tissue sections	
Rat Purkinje cells (cerebellum) Mouse anterior horn motor neurons	12.0 ± 1.5 cells/min 12.0 ± 1.5 cells/min
Cell collection speed (cells/minute) from adherent cell cultures	
SH-SY5Y human neuroblastoma cell line Chinese hamster ovary cells (CHO)	Up to 25 cells/min Up to 25 cells/min

* - acquisition volume depends on the DCU ID and sample viscosity

10 Warranty and Liability

NeuroInDx, Inc. warrants its products to be free of defects in materials and workmanship for a period of one (1) year from date of purchase unless otherwise specified at time of purchase. The foregoing warranty of NeuroInDx, Inc. shall be of no force and effect if buyer has modified or damaged the product. No other warranties of any kind, expressed or implied, are provided by NeuroInDx, Inc. NeuroInDx, Inc. shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products.

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