# BASi > IN VITRO MICRODIALYSIS APPLICATION NOTE

## > INTRO

Microdialysis is a sampling technique used to monitor the chemical composition of interstitial fluid from specific tissues. Microdialysis relies on a diffusion gradient of low molecular weight compounds from the extracellular space across the dialysis membrane. This gradient is established via the continuous perfusion of medium (aCSF) through a semi-permeable microdialysis probe.

Generally speaking, the concentration of analyte in the sample will not be the same as the concentration in the dialysis target, but it will be some fraction of that concentration in the dialysis target. This fraction is expressed as a percentage and referred to as "Relative Recovery." Recovery is variable and is dependent on the membrane type and length, as well as flow rate, analyte properties, temperature and other factors. It is recommended to determine relative recovery of analytes in vitro prior to in vivo experiments for the following reasons:

1. Test the viability of a probe and make sure that the instruments are functioning properly
2. Determine if microdialysis is the appropriate technique for a compound not previously studied
3. Determine the ability of a drug to cross the membrane in preparation for administration through the microdialysis probe

## > METHODS

### Preparing the microdialysis probe for in vitro use:

1. Soak Flanged Tubing Connectors (MD-1510) in 70% EtOH for 10 minutes or longer.
2. Prefill a 1.0mL Gas-Tight Syringe (MDN-0100) with perfusion fluid (MD-2400 or Ringer's Solution) and insert into BASi Infusion Pump. ***Please note:*** *Perfusion fluid should be at physiological temperature.*
3. Run the Infusion Pump at 10-20uL/min to make sure that liquid leaves the tip of the syringe needle.
4. Use soaked Tubing Connectors to connect FEP Teflon Tubing (MF-5164) to the gas-tight syringe.
5. Run the Infusion Pump at 10-20uL/min to check for flow and air bubbles. Once flow has been established and air bubbles have been cleared, stop the Infusion Pump.
6. Connect FEP tubing using Tubing Connectors from the syringe to the inlet port on the microdialysis probe (yellow).

## > MATERIALS

### INSTRUMENTS

#### INFUSION PUMP

| | |
|---|---|
| **MD-1001** | Syringe Drive |
| **MD-10** | Worker Bee Controller |
| **MD-1020** | Bee Hive Controller |

#### IN VITRO CALIBRATION STATION

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| **MD-1522** | Calibration Station with Clamps for BR Style Probe |
| **MD-1524** | Calibration Station with Clamps for MBR Style Probes |

#### AUTOMATED FRACTION COLLECTOR

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|---|---|
| **MD-1201** | Refrigerated Fraction Collector |
| **MW-2310** | Fraction Collector Needle |

#### MICRODIALYSIS PROBES

See all options on reverse side

### ACCESSORIES

| | |
|---|---|
| **MF-5164** | FEP Teflon Tubing (or your preferred tubing) |
| **MD-1510** | Flanged Tubing Connectors (alternatives available) |
| **MD-2400** | Artificial Cerebrospinal Fluid |
| **MDN-0100** | 1.0mL GasTight Syringe |
| **MF-5271** | 250uL Polyethylene Sample Vials (or your preferred vials) |

### OTHER

Mixed standard dissolved in perfusion fluid at a known concentration

Analytical Detection System such as the ALEXYS Neurotransmitter Analyzer from Antec Scientific

7. Run the Infusion Pump at no more than 5uL/min to check for flow. Once flow has been established, stop the Infusion Pump.
8. Connect FEP tubing using Tubing Connectors to the outlet port of the microdialysis probe (green) and place excess tubing into a collection vial (MF-5271, MF-5270).
9. Run the Infusion Pump at no more than 5uL/min to check for flow and air bubbles. Once flow has been established and air bubbles are cleared, stop the Infusion Pump.
10. Run the Infusion Pump at 2uL/min for 30 minutes to flush the glycerol from the probe membrane.
11. The probe is now primed and must remain wet for use.

## IN VITRO RECOVERY

1. Insert two polyethylene vials filled into the BASi Calibration Station (MD-1522, MD-1524). Fill the vials with the mixed standard solution. ***NOTE:*** for best results, keep the fluid at animal body temperature, and take care to ensure that your standard solution remains homogenous.
2. Mount the primed microdialysis probe to the probe clamp on the calibration station, and lower the clamp to ensure that the membrane is submerged in the liquid.
3. Check to ensure that all tubing connections are secure.
4. Set the flow rate of the Infusion Pump.
    a. If the flow rate for you application is known, and you are simply using this study to determine whether the probe is functional for your analyte, then a single flow rate is sufficient.
    b. If this step is being used to determine the optimal flow rate for the in vivo application, then a common approach is to choose three flow rates, for example 0.5uL/min, 1.0uL/min and 2.0uL/min. ***NOTE:*** a lower flow rate will typically result in better recovery, however higher flow rate can make it possible to perform more frequent sampling while still ensuring enough fluid for your analytical system.
5. Collect 3-5 samples per each change to the flow rate (if applicable). Samples can be manually collected into sample vials or automated using the BASi Fraction Collector (MD-1201).
6. Analyze the sample using an analytical instrument (i.e., ALEXYS Neurotransmitters SCC base, part no. 180.0091UW, Sencell 2mm GC sb, part no 116.4120 and analyte specific column) and compare to known standard solution. The simplest way to determine recovery is to equate it with extraction efficiency according to the equation: Extraction Efficiency = Dialysate Concentration/Standard Concentration.

## BASI OFFERS A VARIETY OF PROBES TO MEET YOUR RESEARCH NEEDS

### BRAIN "BR" MICRODIALYSIS PROBES

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|---|---|
| **MD-2202** | BR-1 Brain Microdialysis Probe |
| **MD-2200** | BR-2 Brain Microdialysis Probe |
| **MD-2203** | BR-3 Brain Microdialysis Probe |
| **MD-2204** | BR-4 Brain Microdialysis Probe |

### BRAIN "MBR" MICRODIALYSIS PROBES

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|---|---|
| **MD-2211** | MBR-1-5 Brain Microdialysis Probe |
| **MD-2212** | MBR-2-5 Brain Microdialysis Probe |
| **MD-2232** | MBR-2-10 Brain Microdialysis Probe |
| **MD-2234** | MBR-4-10 Brain Microdialysis Probe |