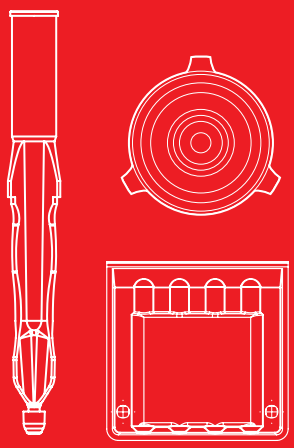
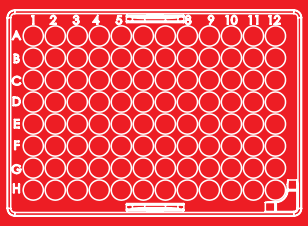
Mitra™
MICROSAMPLING DEVICE





PATENT PENDING

Mitra (RUO) **Microsampling Device** User Manual

Mitra (RUO) is developed and manufactured by Neoteryx. Mitra (RUO) is distributed and supported by Phenomenex. The Mitra (RUO) Microsampling Device is for research-use-only (RUO) and should serve no medical purpose. It should not be used with the intent or in the course of clinical diagnosis, treatment, or care of humans or animals.

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Mitra™
MICROSAMPLING DEVICE

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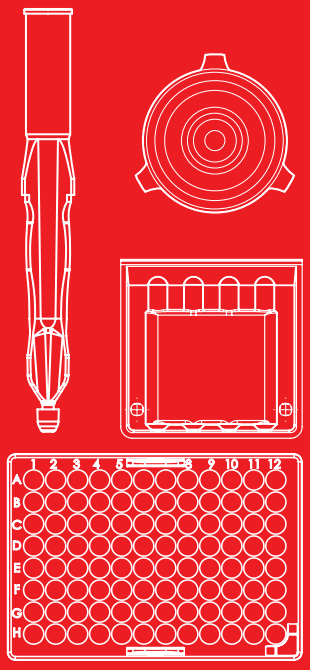

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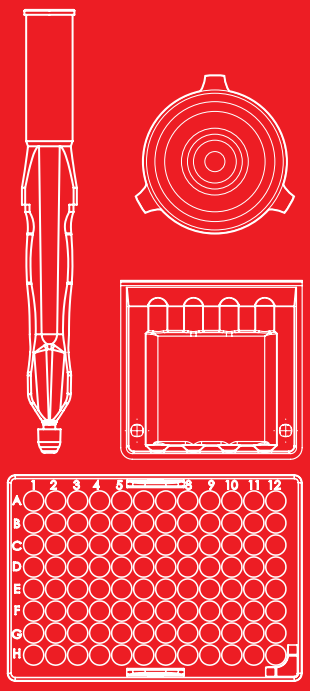
MICROSAMPLING DEVICE

OVERVIEW



The patent-pending Mitra (RUO) Microsampling Device is based on revolutionary, yet simple Volumetric Absorptive Microsampling (VAMS™) technology for the **collection, transport, storage, and analysis of biological fluids**. In seconds, the Mitra Microsampler collects 10 µL resulting in accurate and precise quantitative data while reducing or **eliminating volumetric blood hematocrit bias**. The Mitra Microsampler is dried, stored, and transported **under ambient conditions**. Once ready for analysis, a **simple and automatable extraction procedure** is performed.

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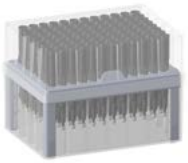
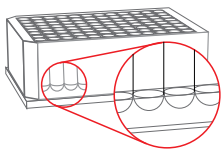
FORMATS & ACCESSORIES

Product	Part No.	Description	Unit	Typical Uses
	9R-K002-CA	Mitra (RUO) 10µL Microsampling Device 96-Well Plate Assembly	1 ea	For high-throughput environments (e.g. discovery labs) For animal studies in drug development For method development
	9R-K002-CD	Mitra (RUO) 10µL Microsampling Device 96-Well Plate Assembly	12/pk	
	9R-K002-BF	Mitra (RUO) 10µL Microsampling Device 4-Pack Clamshell	60/pk	For sample collection in clinical trials, including: Direct Sampling At-home Sampling Remote Locations
	AK-9268-A	Mitra Drying Rack 96-Well Plate	1 ea	To organize sampling for high-throughput environments
	AK-9269-A	Mitra SBS Deck Adapter 96-Well Plate	1 ea	For mounting 96-well plate on deck of liquid handlers
	AK-9270-A	Mitra Sampling Tool	1 ea	To easily manipulate and transfer samplers in 96-well plate assembly
	AH0-8636	96-Well Collection Plate 2 mL round well with round bottom	50/pk	To perform extractions prior to analysis

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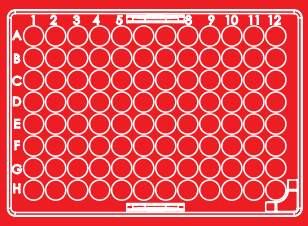
Mitra MICROSAMPLING DEVICE

GENERAL USE PROTOCOLS

96-WELL PLATE



To view protocol demonstration videos visit:

www.phenomenex.com/mitra

Materials

- Mitra (RUO) 10 µL Microsampling Device
- Mitra Drying Rack (recommended for sample organization)
- Mitra Sampling Tool (recommended for sampler handling)
- Mitra SBS Deck Adapter (recommended for automation)
- 96-Well Collection Plate (recommended for extractions)

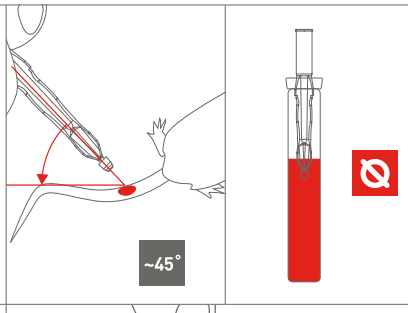
Collection

1. Label sampler bodies and outer box according to your needs/protocol.

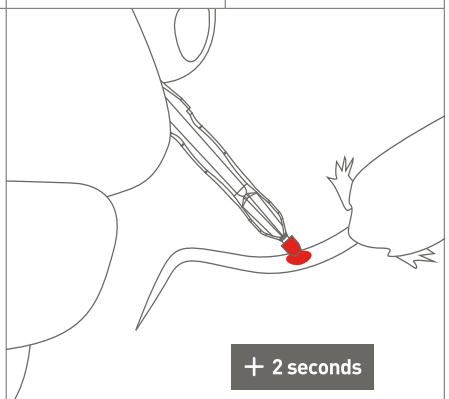
2. Remove rack of 96 samplers from outer box by pinching the two raised tabs on the rack, rocking gently to release, and lifting out (gripping samplers also aids in removal).

3. Remove individual sampler from rack with Mitra Sampling Tool.
- See page 7 of manual to see Sampling Tool use instructions

4. Apply tip of sampler to surface of blood sample. Ensure ~15 µL of blood is available per sample to avoid under sampling.
- Try to sample from a 45° angle for best results, not perpendicularly
- Do NOT fully immerse tip
- Do NOT make contact with skin surface
- Do NOT drip blood onto the tip if sampling from a cannula



5. Once the sampler tip is applied, look for it to go fully red. This will happen VERY fast. Do not get surprised and yank the sampler tip away. Once you see the sampler tip go fully red count an additional 2 seconds and then SLOWLY and SMOOTHLY remove sampler tip from the blood.
- Total contact time for the sampler tip to blood will typically not be longer than 6 seconds (unless hematocrit of the blood is > 60%)
- You will not over sample if sampler tip is left in blood longer than 2 additional seconds as long as it is not immersed. 3 or more seconds is better than stopping too early to ensure sampler tip is full



6. Transfer full tip to drying rack. Be careful not to touch it to the sides of the rack. A simple way to do this is to place the sampler almost vertical to the hole you want to place it in and guide straight down.

7. Repeat steps 3-6 for the remainder of the sampler tips.

Drying, Storage, & Transportation

1. Allow tips to dry in 96-sampler dry rack at ambient temperate for ~2 hours.
- Air should freely circulate around tips to aid in drying

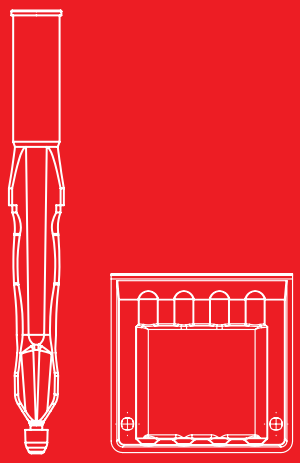
2. Once tips are fully dry (~ 2 hours), the 96-sampler rack can be inserted back into outer box and stored for future use or can be processed for analysis.
- See page 9 for extraction recommendations

3. Fully dried tips are not a biohazard and can be shipped without dry ice, hazardous considerations, or special couriers.

Mitra MICROSAMPLING DEVICE

GENERAL USE PROTOCOLS

4-PACK CLAMSHELL



To view protocol demonstration videos visit:

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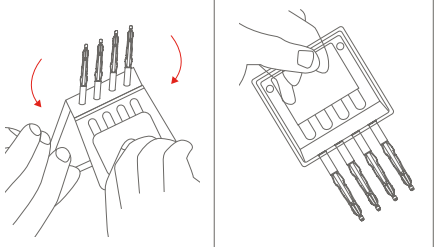
Materials

- Mitra (RUO) 4-pack Clamshell
- 96-Well Collection Plate (recommended for extractions)

Collection

1. Label samplers and clamshell according to your needs/protocol.

2. Uncover sampler bodies by pulling apart clamshell and fold over cover to create a handle for easy sample collection.



3. Apply sampler tip to surface of blood sample. Ensure ~15 µL of blood is available per sample to avoid under sampling.
- Try to sample from a 45˚ angle for best results, not perpendicularly
- Do NOT fully immerse tip
- Do NOT make contact with skin surface
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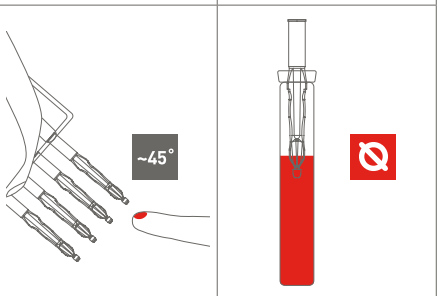

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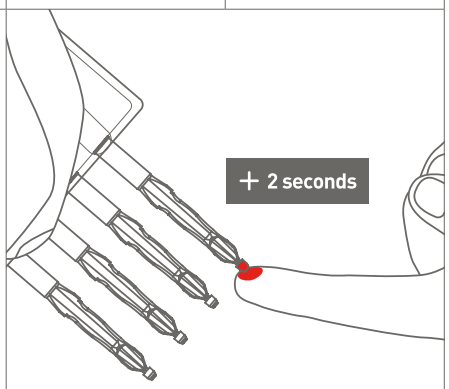


5. Repeat steps 3-4 for the remainder of the sampler tips in the clamshell and then fold clamshell back together and press closed. You will hear it snap into place.

Drying, Storage, & Transportation

1. Closed clamshell can be dried under ambient conditions or immediately placed in a bag with 1 gram of desiccant per sampler.
- IMPORTANT - ensure that the desiccant is fresh or proper drying will not occur and sample may be compromised

2. Once tips are fully dry (~ 2 hours), the samplers can be transported, processed for analysis, or stored for future use.
- See page 9 for extraction recommendations

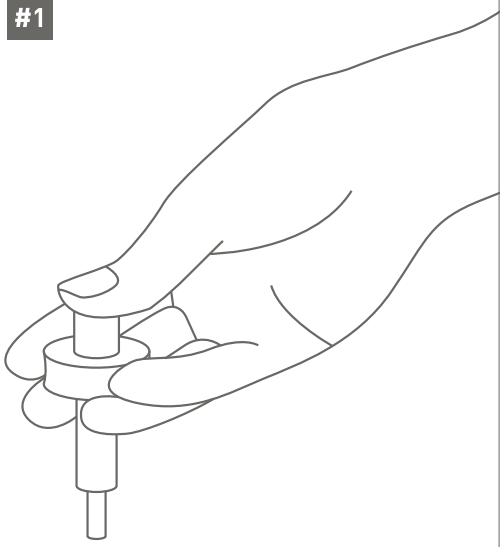
3. Fully dried tips are not a biohazard and can be shipped without dry ice, hazardous considerations, or special couriers.

SAMPLING TOOL USE

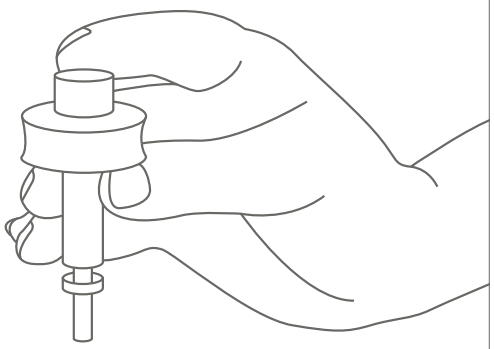
The Mitra (RUO) Sampling Tool assists in picking up the Mitra Samplers from the 96-well drying rack and can also be used to take the sample. There are several ways to hold the sampling tool according to what is most comfortable for you. Below are two recommended holds.

1. Hold Recommendation #1 - Place thumb on top of plunger and grasp the body of the tool between middle and ring finger.
Hold Recommendation #2 - Grasp body of tool between thumb and middle finder and place index finder on top of plunger.

#1



#2



2. Guide tip of Sampling Tool into distal end of Mitra Sampler and press down for a tight fit.

3. Collect blood sample.

4. Press down on plunger to eject Mitra Sampler back into drying rack.

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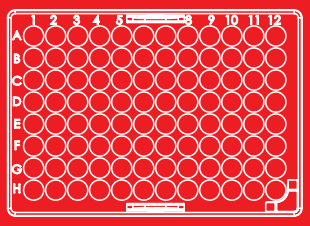
AUTOMATION GUIDANCE

(FOR 96-WELL PLATE FORMAT)



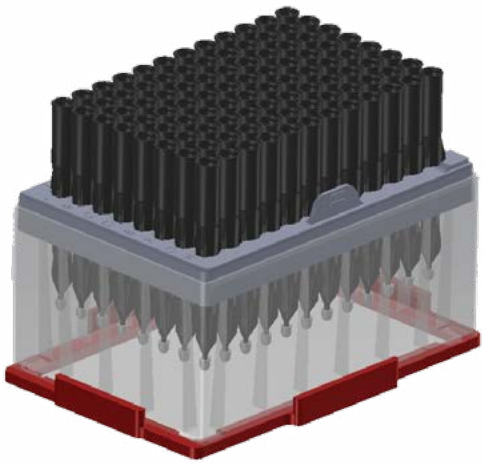
The Mitra SBS Deck Adapter aligns and holds the Mitra 96-well plate assembly to the correct SBS positioning of the sampler bodies to allow automation of sample extraction.

1. Once samples are dry, replace the drying rack holding the dried samples into the bottom portion of the storage box.

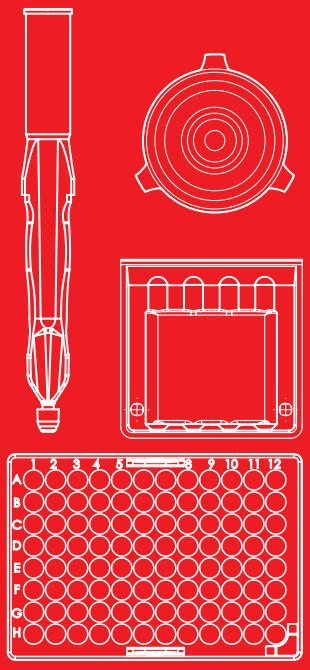
2. Place the adapter plate(s) in the desired locations on the liquid handler deck.

3. Place the Mitra 96-well assembly (drying rack inside storage box) on the adapter plate in the desired orientation.
• The Mitra drying rack has a raised corner for rapid identification of rack/plate orientation

IMPORTANT - The Mitra drying rack should be contained within its storage box when used with a liquid handler and placed on top of the adapter. The storage box, itself, is NOT SBS compatible and should not be used directly on a liquid handler.



EXTRACTION RECOMMENDATIONS



Extraction of the Mitra Sampler is quite simple and very similar to common methods used with dried blood spotting cards. The recommended extraction plate (P/N: AH0-8636) is designed to specifically hold the Mitra Samplers. If desired, the Mitra Sampler fits a standard 20-200 µL pipette head and a liquid handler robot properly equipped to perform the protocol can be used. (see page 8 of manual for automation guidance).

Common extraction solvents recommended include water, methanol, acetonitrile, and ethyl acetate, or mixtures thereof. Below are tips to aid you in extraction method development.

- Neutralizing ionizable analytes (i.e. addition of 1% formic acid for extraction of acidic analytes or 1% of ammonium hydroxide for bases) can improve recoveries
- For high log P analytes the use of lower polarity solvents such as ethyl acetate is advantageous
- Follow water extractions with an appropriate liquid-liquid extraction (e.g. ethyl acetate or dichloromethane (DCM). All extractions using more than 30% water will require centrifugation for the removal of solids
- 2-step extractions such as water followed by acetonitrile can be used
- Alternative methods of physical agitation such as longer vortexing steps, sonication, or heated solvents can be used to improve recoveries

Example - Methanol (MeOH) Extraction

1. Add MeOH to extraction plate (Acceptable volumes are of 200 - 500 µL). We also recommend adding the internal standard at this point.

2. Add Mitra Samplers to the extraction plate.

3. Vortex extraction plate for 15 minutes at 1100 RPM using a platform mixer/rotator.

4. Remove Mitra Samplers from the extraction plate.

5. If particulate is present, centrifuge the Extraction Plate at 1500 G for 5 minutes.

6. Remove supernatant from extraction plate and transfer to a suitable container (e.g. another extraction plate).

7. Blow down to dryness using N_2.

8. Reconstitute sample with solvent suitable for dissolution of analytes.

9. Analyze extract.

IMPORTANT - Extraction of dried blood from Mitra sampler should be optimized using your target analyte at a concentration of 5x your LLOQ doped into 65% hematocrit blood. Absolute recoveries of > 85% under these conditions will provide the best possible results across a range of analyte concentrations and hematocrit levels.

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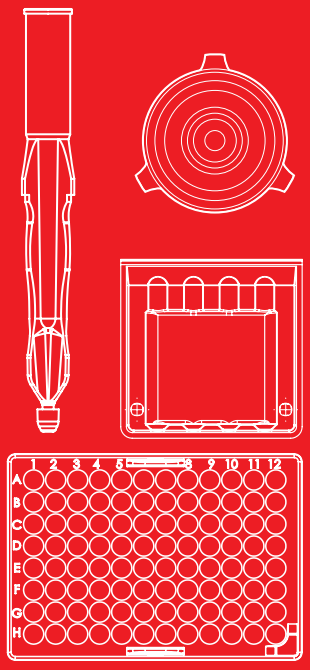
STORAGE & STABILITY



The Mitra Microsampler 96-Well Plate and 4-Pack Clamshell formats should be stored in a dry location under ambient conditions. The shelf-life is 12 months. Parts are labeled with the expiration date.

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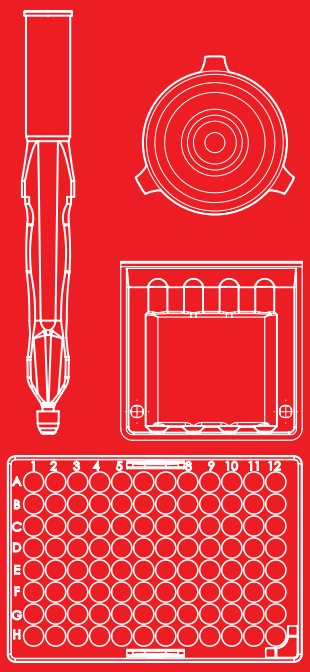
TROUBLESHOOTING



Issue	Most Likely Cause	Solution
Low (<75%) or no recovery of target analyte	A non-optimized extraction method	Optimize extraction method using recommendations found on p.9 of this manual or contact Phenomenex for assistance
Mitra Sampler Tip is not absorbing or taking longer than 6 seconds to fully adsorb	a) Sampler tip is expired b) Sampler tip was stored at a temperature > 110 °F c) Blood has > 65% hematocrit level	a) Use a fresh sampler tip b) Use a fresh sampler tip that has been stored under ambient conditions c) Hold sampler tip to blood pool longer than 6 seconds to allow more time for wicking
Mitra Sampler Tip is not dry after 2 hours	a) Ambient conditions were near 100% humidity b) The samplers in the drying rack were returned to storage box before they were dry	a) Move tips to dry in a low humidity environment or dry under desiccated conditions b) Remove 96-well dry rack from outer box so air freely circulates around sampler tips
Mitra Sampler Tip is not wicking volumes within the specification stated on the Certificate of Conformance	a) Tip was submerged into blood pool b) Did not add the additional 2 seconds once tip went fully red c) Blood has > 65% hematocrit level NOTE - if you are determining the volume by using a density conversion, ensure that the conversion is correct	a) Resample with a new tip and apply it just to surface of blood pool b) Allow tip to have an additional two seconds of contact time with samples c) Hold sampler tip to blood pool longer than 6 seconds to allow more time for wicking
Variation in signal is observed	Mitra Sampler Tip is not fully dry	Tips may appear dry long before they are actually dry. Dry tips longer and try again
Extractions are showing unacceptable variation in response (i.e. > ± 15%)	a) Samples were not completely dry b) Samples did not absorb completely c) Ambient conditions were near 100% humidity	a) Remove 96-well dry rack from outer box so air freely circulate around sampler tips b) Allow tip to have an additional two seconds of contact time with samples c) Move tips to dry in a low humidity environment or dry under desiccated conditions

Mitra™ MICROSAMPLING DEVICE

FREQUENTLY ASKED QUESTIONS

Q: Is there a specific contact angle that the Mitra Sampler Tip should be applied to the blood pool with?

For best sampling performance we recommend sampling from ~45° angle.

Q: Can other fluids be collected using the Mitra Microsampler?

Yes. Other biological fluid (urine, ocular, cerebral spinal, etc.) can be collected as long as there is at least a 20µl pool to collect from.

Q: Can the Mitra Microsampler only be used to collect samples from a live host?

No. Samples can be collected directly from a live host or from blood collected in another device (e.g. Vacutainer®, Hematocrit tube, etc.).

Q: Is there a correct way to add an internal standard?

We have had much success with adding the internal standard as part of the extraction solvent.

Q: What happens if samples are transported before they are fully dry?

If the desiccant that they are transported with is functioning the samples will dry during transport, however samples need to be dried completely before transport or they may be considered a biohazard.

Q: What is the best way to label the 4-pack clamshell and the individual samplers?

For the clamshell, we recommend applying a label to the large, flat square area on the front or back. For the sampler, we suggest applying a label to the distal end.

Q: Can I take a sampler out of the 4-pack clamshell to sample?

Yes. Individual samplers can be removed from clamshell to sample if required. To replace into clamshell after sampling snap back into place.

Q: Is there a limit to how long I can store the tip after the sample has been collected?

This will depend on your analyte, method of storage, and method of extraction. You will need to determine the conditions required for keeping your assay within validation criterion.

Q: Will the tip's color change after extraction? Is the change in the color of the tip an indication of the recovery?

The color of the tip will change depending on the extraction solvents used. It is an indicator that an extraction has occurred. However, it is not indicative of the percent recovery. If the tip is extracted with aqueous it becomes whiter. If the tip is extracted with aqueous and a modifier it will become completely white. If the tip is extracted using an organic solvent, such as methanol, the tip will become brownish.

Q: When collecting blood from a live host, should I wipe the first drop of blood before sampling?

It depends on your sampling protocol. However, it is typically best practice to wipe the first drop of blood before taking the sample.

Q: Will the sampler tip wick at the same speed for all percentages of hematocrit blood?

No. The sampler tip will wick more slowly with higher percentages of blood hematocrit.

Q: Are there any precautions I need to take to make sure the clamshell doesn't open during shipment?

No. The clamshell has been designed with a tight fit and has been tested to not open during shipment.

Q: What happens if I contaminate the sampler body with blood?

This should not negatively affect the results as only the sampler tips will be immersed in the extraction solvent.

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