

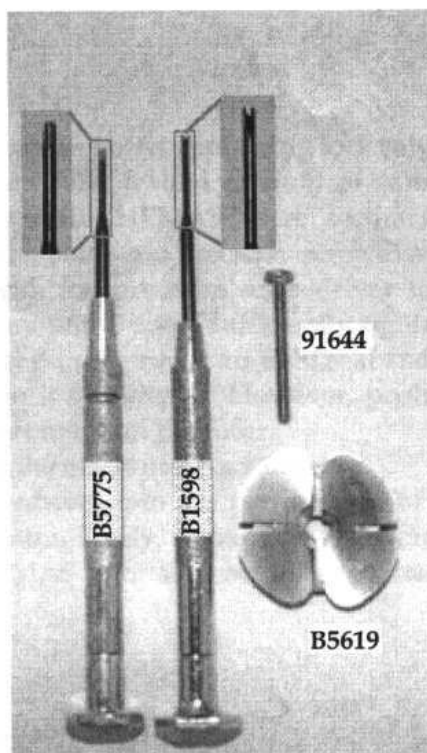


Assembly Instructions for Disposable HRMAS Insert P/N B5866

Instruction for Part Numbers: B3793, 4493, 4494, 4495, 4496

Tools required:

Modified precision screwdrivers with blunt-end (P/N B5775) and forked-end (P/N 1598), HRMAS rotor opening tool (P/N B5619), and insert extraction screw (P/N 91644).

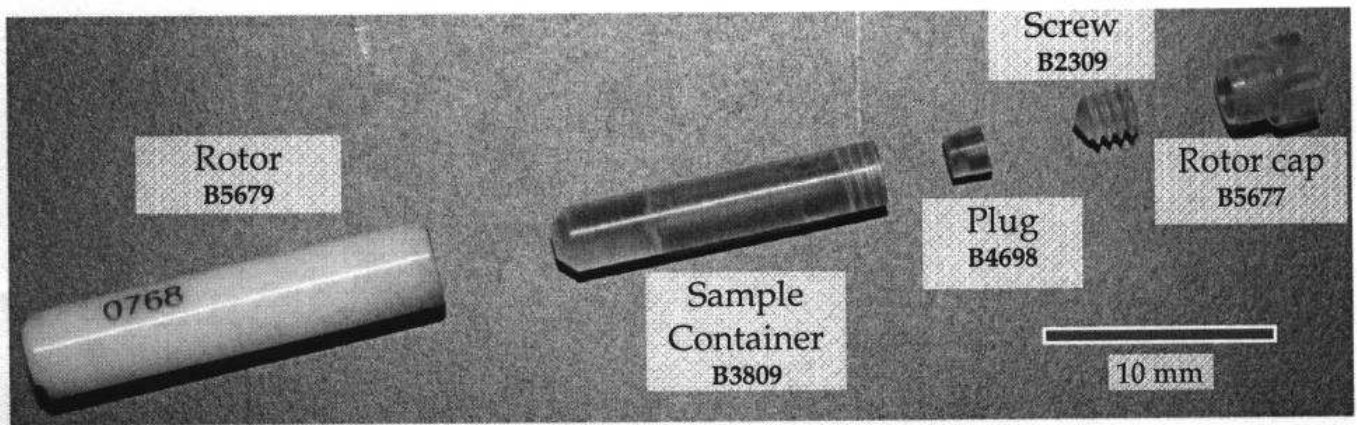


HRMAS Insert Description:

The disposable HRMAS insert has three parts: the sample container (P/N B3809), a plug (P/N B4698) to close the container, and a screw (P/N B2309). The insert is made of biologically inert Kel-F®. The sample container holds approximately 30 μ L of liquid. The insert plug seals the sample inside the sample container. The top surface of the plug has a groove which fits into the blunt-end precision screwdriver which facilitates in fitting the plug into the sample container. The plug restraining screw holds the plug in position. The assembled insert fits into a standard Bruker 4 mm zirconium rotor

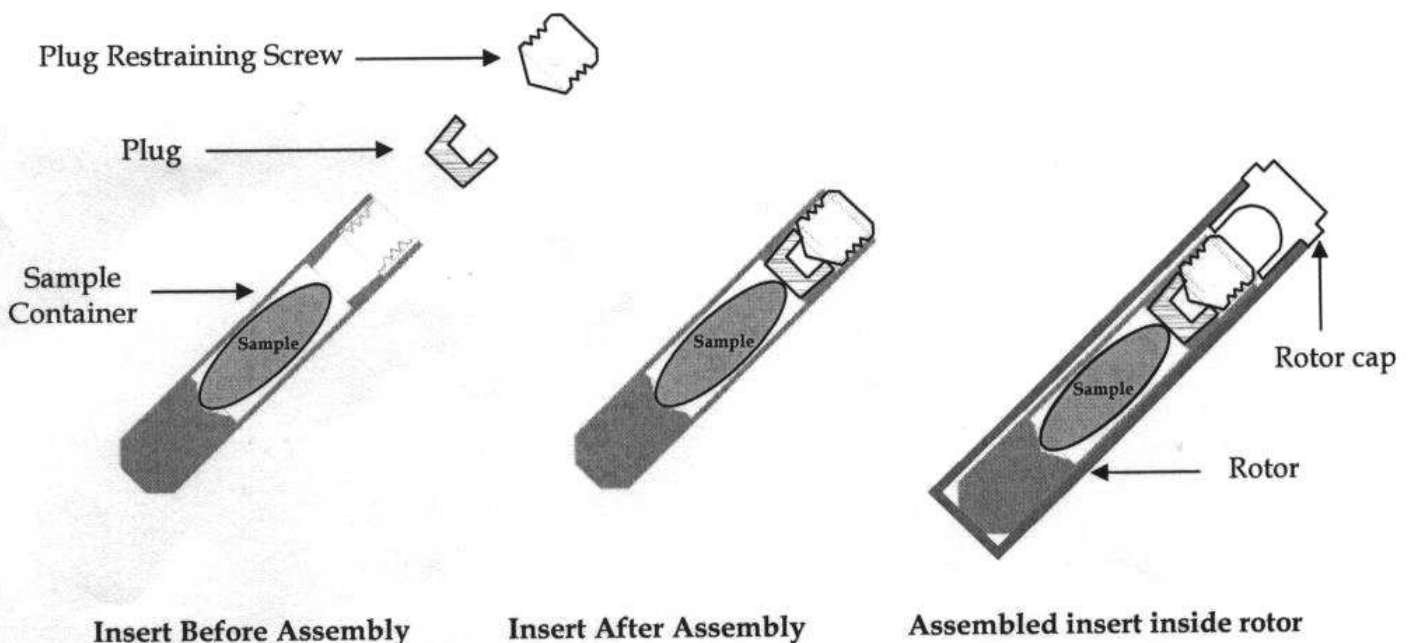
Recommended Spin Rate: 4 kHz
Maximum Spin Rate: 6 kHz

Disposable HRMAS Insert Operations



Sample Loading:

1. Add 5-10 μL of lock solvent or buffer containing lock solvent into the sample container.
2. Fill the sample container with desired amount of sample (25 - 30 mg). See "Tissue Sampling for Bruker Disposable HRMAS Insert" section for details.
3. Fit the insert plug onto the blunt-end precision screwdriver.
4. Press the insert plug with the precision screwdriver to securely fit it into the sample container. The correct amount of force sufficiently tightens the plug without expanding the insert wall. The insert plug forms an air tight seal and prevents sample leakage; hence it is important to tighten it sufficiently. However, pushing the plug too much into the insert will cause the insert not fit in the rotor.
5. Wipe any liquid outside the insert using a Kimwipe.
6. Fit the forked-end screwdriver into the perforations of the plug restraining screw and screw it until it stops rotating freely. Avoid over tightening.
7. Load the insert into HRMAS rotor as described in "Loading the insert into the HRMAS rotor" section.



Loading the insert into the HRMAS rotor:

1. Put the insert assembly into the HRMAS rotor.
2. Using the forked-end screwdriver, gently press the insert until it is completely in the HRMAS rotor.
3. Close the rotor with the rotor cap.

Retrieving the insert from the HRMAS rotor:

1. Remove the rotor cap using the HRMAS rotor opening tool.
2. Unscrew the plug restraining screw.
3. Screw the insert extraction screw into the HRMAS insert.
4. Remove the insert from the rotor.

If you are not able to unscrew the plug restraining screw due to friction-less rotation of the whole insert assembly,

1. Insert the prongs of the forked-end precision screwdriver into the perforations of the HRMAS insert screw.
2. With the screwdriver, carefully remove the insert from the rotor by gently pressing it against the side of the rotor and pulling it up.

Freezing HRMAS Insert with Sample:

Owing to small size of the insert and insert components, the expansion of water during its freezing process may deform the insert. A deformed insert may not fit into the rotor. However, the insert deformation has been observed to be sample dependent. So, it is important to test if your sample causes deformation of the insert after a freeze-thaw cycle. If you observe deformation of the insert after a freeze-thaw cycle for your sample, unwind the plug restraining screw by half-a-turn (180°) before freezing the sample with the insert. After thawing the sample and the insert, rewind the screw back by half-a-turn.

Alternatively, the inserts can be frozen with the samples in a Bruker 96-slot HRMAS insert storage rack (P/N B5771). The slots in the rack are designed to restrict horizontal expansion of the insert during freezing. The slots also act as a gauge which determines if the insert will fit into the rotor.

Tissue Sampling for Disposable Bruker HRMAS Insert

Tissue sampling for HRMAS insert require special tools and skills due to its relatively small size. At room temperature, most tissues have semi-solid consistency which is not ideal for efficient sampling. Moreover, tissues degrade rapidly at ambient temperature. For best results, sample tissues when it has a rigid consistency. For most tissues (e.g., liver, kidney, brain, muscle, etc), this is achieved by freezing the tissue below -20 °C. Maintaining the temperature of the tissues below -20 °C during sample manipulations also helps to reduce the amount of sample degradation. The sampling/cutting operations on the frozen tissues are ideally performed in a cold room or on a cold block (< -20 °C) to prevent rapid thawing of the sample.

The disposable biopsy punches, dermal curettes, and scalpels are very useful for sampling and transferring tissues into HRMAS inserts. The disposable nature of these tools enables the processing of large numbers of samples without cross contamination. Biopsy punches are useful for reliable, fast, and efficient sampling of frozen tissues. The 2 mm biopsy punches were found to be ideal to use with Bruker HRMAS inserts. The 1.5 mm biopsy punches are useful to obtain smaller volumes of tissues. Curettes are useful when a thin slice of sample (0.5 – 2 mm) is required from the surface of a tissue (useful for investigating multi-compartmental organs such as kidney, eye, etc.). A scalpel is generally useful to cut tissues any desirable sizes. It may be particularly useful in cutting tough tissues such as tendons and ligaments.

Tools and consumables:

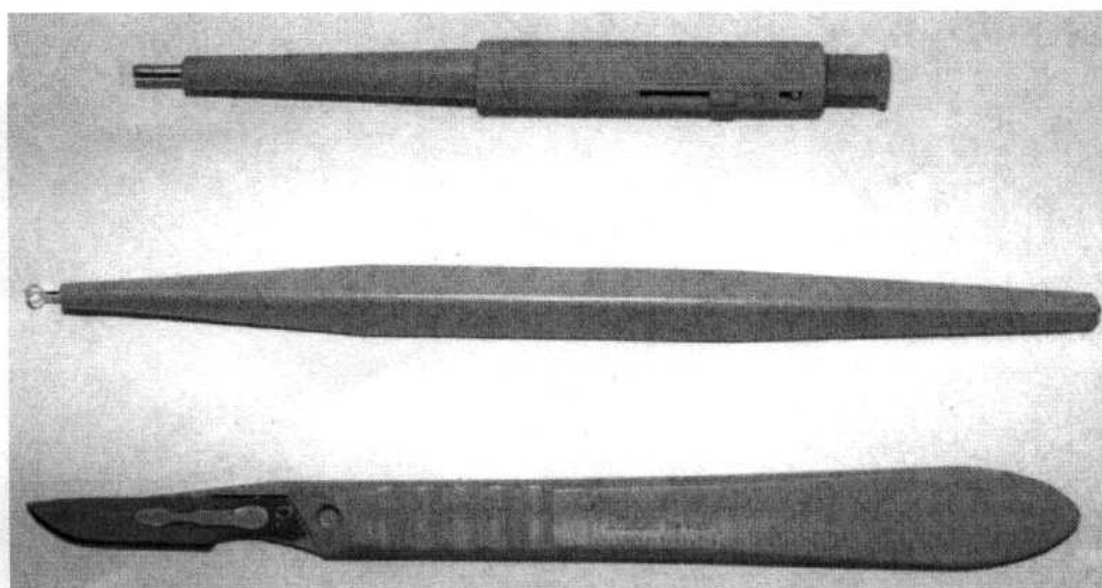
HRMAS insert assembly (Part Nos. B3793, 4493, 4494, 4495, 4496)

Disposable Biopsy punches - 3 mm (Part # 93384)
2 mm (Part # 93383)
1.5 mm (Part # 93385)

Disposable Curette (Part # 93414)

Disposable Scalpels (Part # 93386)

Note: The disposable biopsy punches, curettes, and scalpels supplied by Bruker are not intended for human use.



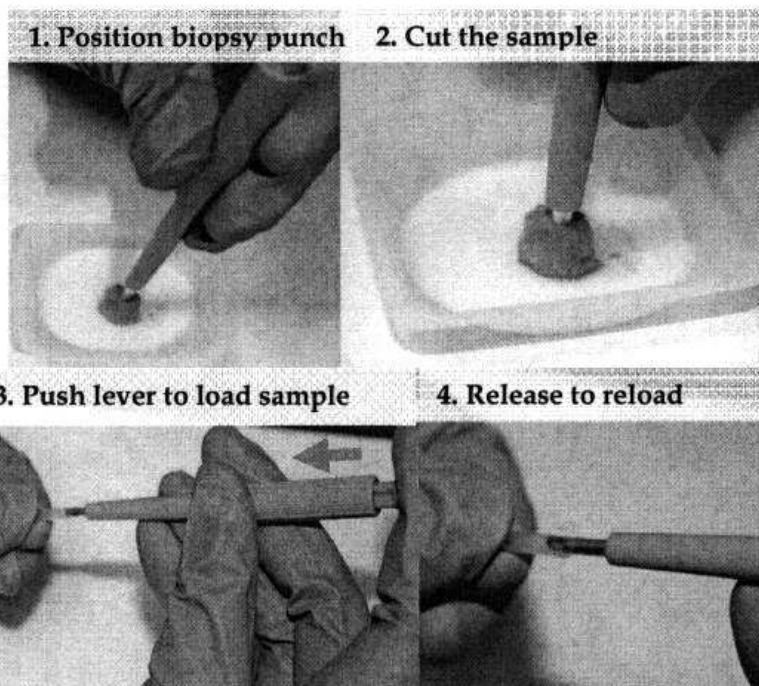
Biopsy punch
93383

Curette
93414

Scalpel
93386

Procedure:

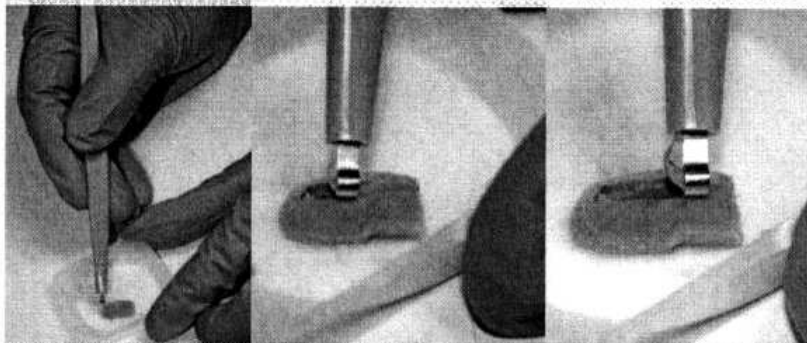
1. Add required amount of buffer into the HRMAS insert (5-15 μL). Weigh the HRMAS insert, insert plug, and plug restraining screw together to get the weight of the fully assembled insert without the sample (important for quantitative studies). Due to the small size of the insert, use of a weighing balance with a precision of at least ± 0.1 mg is recommended in order to obtain quantitative results with good precision. When working with biohazardous samples, weighing the inserts in a clean, labeled, 0.65 mL microcentrifuge may reduce the possibility of contaminating the weighing balance.
2. To perform tissue sampling (to be performed in a cold room or on a cold block):
 - a. Place a clean plastic weighing boat on cold block.
 - b. Place the frozen tissue (small piece may be excised from a larger piece using a disposable scalpel) on the weighing boat.
 - c. When using a Biopsy punch: Push the cylindrical blade of the biopsy punch into the tissue area where you wish to sample. Hold the sample and make a circular motion (at 15-30° angle from the line of initial biopsy incision) to cut the sample from the bulk.



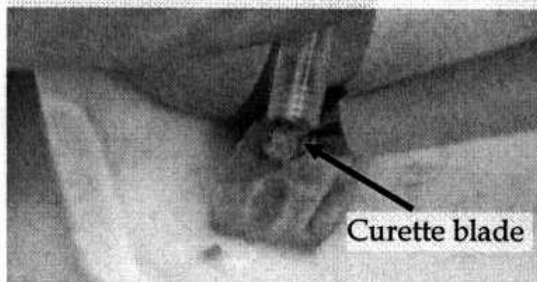
- d. When using a Curette: Push the curette ring in to the tissue to desired thickness and gently grate the frozen tissue. Collect the excised tissue emerging out of the curette ring with the HRMAS insert. When desired length of tissue is cut, excise the sample by moving the curette upward (scooping motion).

1. Position curette

2. Grate sample to appropriate length/depth

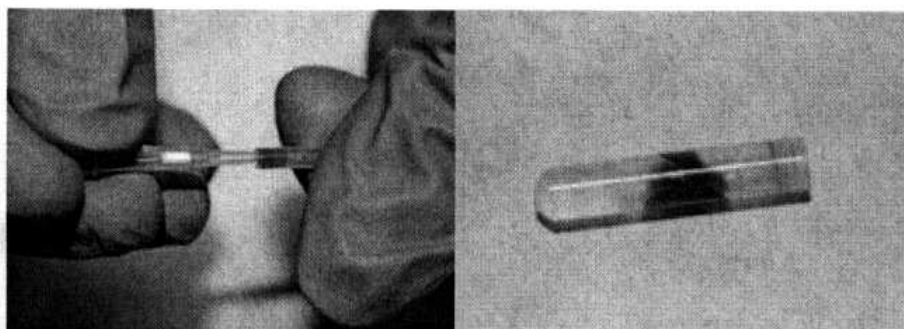


3. Collect sample with insert and excise by scooping up



e. When using a Scalpel: Cut the tissue sample to a dimension that would fit into the insert using a scalpel.

3. Load the sample into the insert. If you need more sample, repeat steps 1 and 2. However, it is important to minimize the number of sample cutting manipulations when filling the insert. This reduces the amount of tissue-degrading enzymes that are released from the ruptured cells. Use a toothpick, pin or pipette tip to push the sample inside the insert or put the insert into a microcentrifuge tube and spin it for 10 s at 1000g in a centrifuge.



4. Close the insert with the insert plug (see HRMAS insert assembly instructions). Wipe any excess liquid with a Kimwipe and put the plug restraining screw. The exterior of the insert may be wiped with ethanol for decontamination. If this decontamination procedure is used, be sure to fully dry the exterior. Note: Optimizing the amount of buffer and sample added into the insert will help in reduction of liquid loss when closing the insert. Liquid loss in this step will lead to underestimation of the actual sample weight.

Put the insert in the labeled microcentrifuge tube and reweigh the insert to obtain sample weight.

Assign a slot for the insert in the Bruker 96-slot HRMAS insert storage rack (P/N B5771) for efficient tracking of the sample. Store the samples in the rack at -80 °C until data collection. The Bruker 96-slot HRMAS insert storage rack is designed to reduce any insert deformations due to water expansion during freezing.

