DiATOME

Diamond Knives

Handling and Use

distributed by

SCIENCE SERVICES

Science Services GmbH
Unterhachinger Str. 75 · 81737 München / Deutschland
T: +49 (0)89 18 93 668 0 · F: +49 (0)89 18 93 668 29
Info@ScienceServices.de · www.ScienceServices.de

Handling and Use

Introduction

Dear customer,

Diatome is the leading manufacturer of diamond knives for ultramicrotomy in biolocical and materials research, with many years experience. The expertise obtained in collaboration with our customers enables us to offer you the highest quality knives available in the market today. This manual was written in order to allow you an easy handling and use of our knives. Please contact us, if you should need any assistance or if you have any special requirements in your ultramicrotomy applications. We are only a phone call or an email away and look forward to hearing from you.

Sincerely,
The Diatome Team

Handling

A diamond knife is not as fragile as you might think. Taking into account the vast array of soft and hard specimens it cuts successfully, the diamond knife cutting edge is quite durable.

With proper care and handling, a Diatome knife can withstand a great deal of use. However, in order to avoid any unfurtunate mishaps, the following precautions should be taken when receiving the knife:

- Inspect the two securing stickers on the knife box and inform us if they have been cut or damaged.
- Ask your receiving department not to open the box.
- When unpacking the knife, care should be taken not to touch the cutting edge.
- Once the knife is unpacked it is ready for use and does not need initial cleaning.

Preparation before sectioning

A perfect section ribbon can only be obtained from a well trimmed block. The upper and the lower side of the block must be parallel to the knife edge. The block should not be too wide because this would substantially increase the cutting pressure. This may result in «chatter».

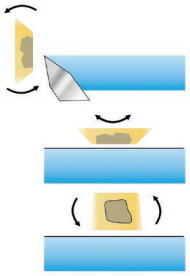
We have found that the best trimming results of biological and materials research samples, at room- and cryo temperatures, are obtained with our diamond trim blades trim 45 (ord. no. DTB45), trim 20 (ord. no. DTB20), and trim 90 (ord. no DTB90). See our knife brochure for more information.

Caution
If trimming is done with a razor blade, always use a fresh, degreased blade of good quality. If the razor blade is old and damaged, it has tendency to leave steel particles on the sample block. Upon sectioning, these particles will cause damage to the diamond knife's cutting edge. If a sample block is precut with a glass knife, use only a clean new portion of the cutting edge. This will reduce the risk of glass particles sticking on the sample block. Avoid during the entire preparation of the sample blocks the embedding of hard particles from pipettes, razor blades, glass knives, etc.

Sectioning at room temperature

The quality of sections will be determined by a number of factors. The following points should be checked before commencing sectioning:

- Set the clearance angle and the cutting speed as indicated on the guarantee card.
- Tighten all of the screws in the sample block holder, the knife holder, etc.
- Align the block (with the backlight on) parallel to the cutting direction.
- Align the block (with the backlight on) parallel to the cutting edge.
- Align the lower side of the block (with the backlight on) parallel to the cutting edge.



The water in the boat should be level with the cutting edge and give a good reflection. **Exception:** For the sectioning of hydrophilic embedding materials we recommend lowering thewater level to a concave shape.

To avoid difficulties during sectioning and extend the life of a diamond knife, the following should be kept in mind:

- The sample block should be fully polymerized.
- Do not add solvents (i.e. acetone) to the distilled water in the boat. They may dissolve the sealing material between the knife and the boat. If the adding of solvents is unavoidable, ask us for sealing the knife with an epoxi resin.
- Avoid cutting thicker sections than the limits given for each type (ultra 150nm, cryo 500nm, histo 2µm).

- Exercise care when picking-up sections. Do not touch the cutting edge with any solid objects (grids, loops, tweezers, etc).
- Do not allow the sections dry on the cutting edge.

Sectioning at low temperatures

Trimming

For successful cryosectioning of biological and materials research specimens trimming is imperative. Our diamond trim blades trim 45, trim 20 and trim 90 allow you to quickly and easy fulfill your trimming requirements.
For trimming we recommend the same temperatures as set for the sectioning.
For trimming it is advisable to use an ionizer. It eliminates section debris sticking to the specimen and on the trim blade.

Sectioning

For dry cryo sectioning the following temperatures are recommended for the cryo chamber, knife and specimen:
- Sucrose infiltrated biological samples:
  semithin sections – 80°C, ultrathin sections – 110° to – 120°C
- Frozen hydrated biological samples: – 150°C
- Polymers, rubber: – 120°C (or below the glass transition temperature) .

For dry cryo-ultramicrotomy the use of an ionizer allows the cutting of perfect section ribbons and an easy section collection.

For the cryo sectioning with boat liquid (i.e. DMSO/water 50/50%) set the following temperatures:
- Cryochamber – 120°C
- Specimen – 120°C
- Diamond knife – 30°C

These figures usually allow best cutting results. Please contact us for more detailed information.

At low temperature the handling of the diamond knives is the same as for room temperature. Be careful when picking up the dry cryo-sections from the diamond surface. The cutting edge may not be touched with any solid objects (i.e. loops, tweezers or grids)!

Cleaning

Method 1

Immediately after picking up the sections, remove all unused sections with a hair-curl on an eyelash. Then clean the knife edge as follows:

- Empty the boat, dry carefully with filter paper (without touching the knife edge!) and remount it in the ultramicrotome knife stage.

- Take one of our polystyrene sticks and bevel it to an angle of approx. 60° using an oil free razor blade.

- Dip the rod into 100% ethanol and shake off the excess.
- Pass the rod over the cutting edge without applying pressure.

Mechanical cleaning as mentioned above is absolutely necessary after cutting poorly polymerized blocks (i.e. Lowicryl polymerised in a freeze substitution apparatus).

Method 2

If you do not feel comfortable using method 1, there is an alternative.
Immediately after picking up the sections, proceed as follows:

- Remove all unused sections with a hair curl or an eyelash.
- Rinse the knife thoroughly with distilled water.
- Take a can of clean pressurized air and blow the water off the cutting edge.

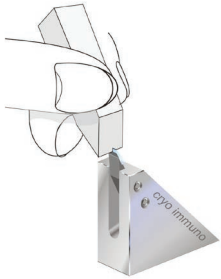
Method 3

If sections or debris dry on the knife edge we recommend the following cleaning procedure:

- Place the knife in distilled water, adding one or two drops of mild liquid dishwashing detergent.
- Let the knife sit overnight.
- Remove the knife and rinse with distilled water.
- Now proceed to clean the knife using method 1.

Cleaning of the cryo diamond knives

- Remove the knife from the cryo chamber (before heating the chamber up!).
- Rinse the knife under tap water to warm it up.
- Clean the knife using method 1, use 50% ethanol for cryo knives used in biological applications, use 100% ethanol for cryo knives used for polymer sectioning.



Caution

We do not recommend sonification for the cleaning of any of our diamond knives. Solvents and acids should never be used under any circumstances.

Resharpening

Diatome diamond knives can be resharpened an unlimited number of times due to special attention given to minimising the amount of grinding during the sharpening procedure. The cutting edge length is not affected.

Our sophisticated manufacturing and resharpening methods are unique and allow us to guarantee that your resharpened Diatome knife will be of the same superior quality as your new Diatome knife.

Guarantee

Before delivery, each knife, new or resharpened, is subject to extensive testing. Only if it's performance is to the highest standards, we will ship ist.

In the unlikely event that you experience difficulties, or for any reason are unhappy with the performance of your knife, contact us immediately. Your short description will allow us to solve the problem to your full satisfaction.