



## Hettich centrifuges in environmental analysis Diatom preparations for water analysis

One of the aims of the EU Water Framework Directive is to ensure that all of the water bodies in Europe have a good ecological quality by 2015. Centrifuges are frequently used in different ways in biological laboratory testing. An example of their use is for the preparation of diatoms.

## Procedure<sup>\*)</sup>:

The water sample is first enriched through centrifugation.

### **Centrifugation parameters for enrichment / purification:**

RCF:	625 (corresponds to approx. 2,000 min <sup>-1</sup> , depending on the rotor)
Running time:	10 minutes
Volume:	50 ml or above
Temperature:	Room temperature

The supernatant is decanted with care and 30 ml H<sub>2</sub>O<sub>2</sub> (30%) added to the pellet. The pellet and H<sub>2</sub>O<sub>2</sub> are left to incubate for several hours (preferably over night). The hydrogen peroxide cleans the silica skeleton of the diatoms so that they are more recognisable.

The H<sub>2</sub>O<sub>2</sub> is then removed by washing. Caution is necessary as it is highly reactive (corrosive and an irritant). The sample is centrifuged again with the same parameters as above. The diatom preparation is washed 4 times in distilled water.

**Important:** Use sealed plastic vessels for centrifugation and ensure that the H<sub>2</sub>O<sub>2</sub> does not come into contact with any metal parts of the centrifuge (this includes H<sub>2</sub>O<sub>2</sub> vapour if possible!). Observe proper safety precautions when handling H<sub>2</sub>O<sub>2</sub>.

After the 4th wash the supernatant is decanted, leaving just a few drops behind. The pellet is resuspended in the water and a drop of the diatom suspension is pipetted on to a cover slip. The drop is air dried and then mounted by turning the cover slip over onto a slide with mountant on it.

## Alternative<sup>\*\*)</sup>: Use of the Hettich cyto accessories

To enable a relatively large volume to be processed (max. 8 ml) to yield a more dense sediment, we recommend the use of our cyto accessories. After the 4th wash step, a larger volume is allowed to remain in the tube (e.g., 10 ml). A cyto insert is then mounted, comprising a commercial microscope slide that has been labelled, a slide carrier and a cyto chamber. The enriched sample is filled into the cyto chamber and the insert placed in the suspension. Caution: Make sure that the rotor is loaded evenly, i.e., two facing buckets at the same time! Centrifugation is now carried out so that the suspended particles in the sample are sedimented onto the microscope slide. After centrifugation the supernatant is decanted and the cyto insert is taken apart. The microscope slide now has a round wet sediment on it, 240 mm<sup>2</sup> in size, which mainly comprises diatoms. This sediment is air dried. The sediment will always be in the same place on a microscope slide and be of the same size - this makes the result more reproducible and easier to evaluate.

### **Centrifugation parameters**

RCF:	225 (corresponds to approx. 1,200 min <sup>-1</sup> , depending on the rotor)
Running time:	5 minutes
Volume:	8 ml
Temperature:	Room temperature

To prevent evaporation artefacts, such as crystals, from developing on the microscope slide it is recommended that the wet cell sediment be dried using a heating plate.

<sup>\*)</sup> Method of Lüttig & Friends GbR, Die Biologinnen, Berlin

<sup>\*\*)</sup> Method developed by Hettich

Almost every Hettich centrifuge can be used for the described application. As a suggestion, we selected a commonly used model that enables both procedures.

## Ordering information

Centrifuge	Cat. No.
ROTOFIX 32 A	1206

Selection of accessories (for the method of Lüttig & Friends)	Cat. No.
4-place swing-out rotor	1624
carrier for tubes up to a volume of 100 ml	1381
lid with bio-containment for carrier 1381	1382
adapter for conical 50 ml tubes with screw cap	1384

**Other tubes require different adapters.  
We will be happy to advise you!**

Selection of accessories (for the alternative method using Hettich cyto accessories)	Cat. No.
4-place swing-out rotor	1624
carrier for cyto suspension, fitting into rotor 1624	1660
slide carrier with fastening ring, fitting into suspension 1660	1662
cyto chamber for a max. sample volume of 8 ml	1666

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