

# FREEZE QUENCH

Compatible with { SFM-20  
SFM-300  
SFM-400



## Fast freeze quench for intermediates and metallo compounds

When chemical quenching is not suitable for a rapid kinetics study, fast freezing the reaction to quench it may be the best option. The freeze quench experiments are divided into three phases: the mixing, the ageing and finally the freezing. The frozen reaction products are collected and analyzed by EPR/NMR, Mossbauer or XAFS in their solid state.

Freeze quench can be used to study full and intermediate kinetics by trapping reaction intermediates whose presence has been determined by stopped-flow experiments. Freeze quench techniques are also extensively used to study of metallic reaction centers in **metalloproteins** and **metalloenzymes**

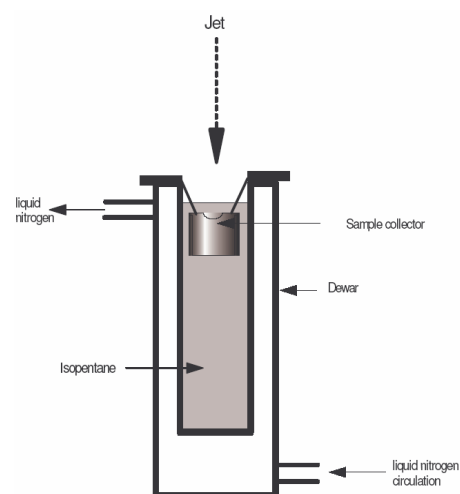
### Unique features

- Independent syringe drives
- Software controlled mixing ratios
- EPR/NMR, Mossbauer, and XAFS sample holders
- User exchangeable with other observation/collection heads
- Integrated ejection nozzle
- Factory calibrated ageing loops

### Principle

The freeze quench accessory consists of an umbilical connector, a set of ageing loops, a diverting electrovalve, and a dewar with a sample holder. Reactants are loaded in the mixer syringes, then the reaction is initiated by mixing 2 or more solutions. The reaction is aged for a user-defined time inside the calibrated ageing loops. An built-in ejection nozzle at the exit of the ageing loop sprays the aged solution towards the dewar. A diverting electrovalve between the nozzle and dewar allows the user to program a pre-washing phase. The dewar is filled with isopentane as the cryosolvent. The temperature is regulated using circulating liquid N<sub>2</sub> in the dewar jacket, or by immersion in a liquid N<sub>2</sub> bath. The sample collector is suspended so it is immersed in the cryosolvent at the top of the dewar. Cryosolvent level is maintained as close as possible to the nozzle to minimize flying time. On ejection the reaction is stopped below -130°C, and the frozen sample is packed directly into the sample holder (NMR tube, EPR tube, etc.). Sample consumption depends on the amount of material necessary for off-line analysis, for example, to get about 1 cm of crystals in an EPR tube, the user needs to collect around 200 µl of solution. Ageing times, including flying time and freezing time, range from 9 ms to several minutes.

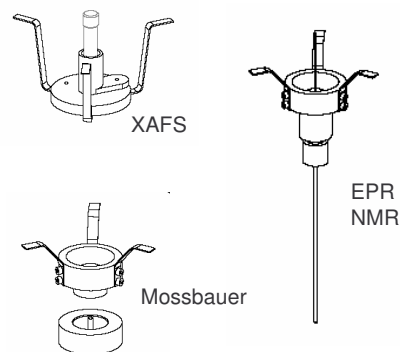
The freeze quench accessory can be exchanged with other stopped flow accessories in minutes.



## Different sample collectors

To limit the number of steps during the packing process and avoid the risk of warming up the frozen solution, aged solution is ejected directly into a cooled Teflon funnel. The sample holder is attached to the bottom of the funnel cone so the sample can be packed easily with the included packing rod. Holders are available to collect samples for spectroscopic analysis by EPR/NMR, Mossbauer, and XAFS. Dewars of different volumes are available to optimize consumption of isopentane according to the type of sample holders being used.

The freeze quench accessory includes a collection kit for one type of sample. Additional kits are available as options.



## Software control

Delay Line 1 (µl)  
 n\*1 (17) 43,7

Delay Line 2 (µl)  
 [ ]

Ejection delay Line (µl)  
 n\*3 93,6  
 n\*1 19,0  
 n\*2 41,6  
 n\*3 93,6  
 n\*4 141,8  
 n\*5 189,5

Bio-Kine32 is used to design the freeze quench sequence and control the mixer. The user selects the volume of the ejection delay line, and ageing time will be calculated in the driving sequence. The user can edit the sequence to create multiphase experiments, including control of the diverting valve to determine if solution is waste or to be analyzed. For ageing times from 8 ms to 200 ms the mixing is done in continuous flow mode, for longer ageing times the system work in a push-pause-push mode.

Quenched Flow Program

|             | Phase 1 | Phase 2 | Phase 3 | Phase 4 | Phase 5 | Total Volumes (µl) |
|-------------|---------|---------|---------|---------|---------|--------------------|
| time (ms)   | 50      | 25      |         |         |         |                    |
| Syr. 1 (µl) | 150     |         |         |         |         | 150                |
| Syr. 2 (µl) | 150     | 150     |         |         |         | 300                |
| Syr. 3 (µl) | 100     | 50      |         |         |         | 150                |
| Valve       | Waste   | Collect | Waste   | Waste   | Waste   |                    |
| Syncho 1    | Off     | Off     | Off     | Off     | Off     |                    |

Phase: 2/5    Total Volume: 200 µl    Total Flow Rate: 8,0 ml/s

Syringes contents:  
 Syringe 1: myoglobin  
 Syringe 2: Det-A  
 Syringe 3: azide

Shots: Single / Multiple  
 Drive Sequence: 1(1:20)  
 Ageing Times: DL1: 7,3 ms, DL2: [ ], EDL: 11,7 ms

## Specifications

|  |                             |
|--|-----------------------------|
| Priming volume of umbilic  | 200 µl                      |
| Temperature control<br>(using temperature regulation circuit of SFM) | Yes                         |
| Volumes of ageing loops  | 19 µl, 41 µl, 93 µl, 190 µl |
| Freezing time (isopentane)   | 3-4 ms                      |
| Minimum flying time  | 1 ms                        |
| Minimum ageing time ( in the ageing loop)                            | 4 ms                        |
| Minimum ageing time (TOTAL)  | 9-10 ms                     |



## Included with FQ accessory

- Umbilical connector
- Set of 4 ageing loops ( from 19 µl to 190 µl)
- Diverting valve
- Dewar and one collection kit to be chosen between EPR, NMR, Mossbauer or XAFS
- Packing rods
- Mechanical adaptor for stopped-flow

## Site requirements

- Liquid nitrogen and circulation system
- Magnetic rod and magnetic stirrer plate
- Isopentane
- Vacuum pump for waste evacuation
- Sample holder ( EPR tube, NMR tube, etc., depending on collection kit chosen)

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