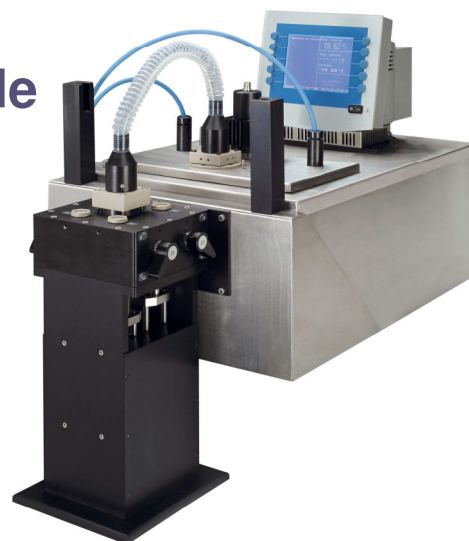


# CS -90 °C

## Liquid N<sub>2</sub> cryo-stopped-flow upgrade

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

The cryo-stopped-flow assembly extends the range of operation of the Bio-Logic stopped-flow instruments to far sub-zero temperatures. It allows Transient States Kinetics experiments to be performed down to -90°C.



### Temperature control

CS -90 °C includes a 22 liters cryo-bath. Isopropanol or ethanol is commonly used as a cryosolvent. The cooling is done by circulating liquid N<sub>2</sub> in a coil immersed in cryosolvent. The temperature regulation is made by creating an equilibrium between a heater and the flow of N<sub>2</sub>. When experimental temperature is reached, flow of N<sub>2</sub> is stopped and temperature is stable during several minutes which gives enough time for tens of experiments. Temperature dependence studies can be done from -90°C to +20°C without any reconfiguration. The limitation is the cryosolvent, in fact by replacing the cryosolvent by oil you can also use the system for High temperature stopped-flow.

### Connection to stopped-flow

The **umbilical connector** allows fluidic connection between SFM and the observation cell. This allows a physical separation of these two parts of the stopped-flow. The mixing unit can be cooled by the cryosolvent while the rest of instrument can be kept at more classical temperature. The fluid used for regulating the stopped-flow temperature also circulated into the umbilical connector keeping the active solutions at a constant temperature until the end of the connector.

The cryo accessory is also equipped with **Isolast o'rings for full solvent compatibility** ( caution: the stopped-flow must also be equipped with similar o'rings).

**CS-90 °C is suitable for single mixing application** but double mixing capabilities is also available in option.

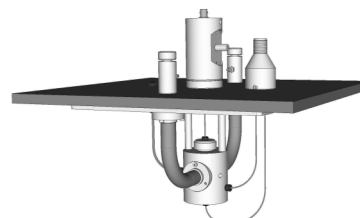
### Detection

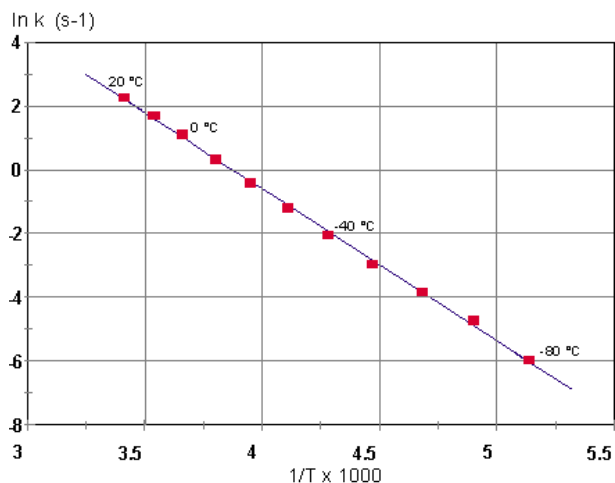
Detection is made through optical fibers which are installed on the cuvette. The optical fibers are protected from cryosolvent by umbilical tubes. It is also possible to flush nitrogen or inert gas through these tubes for anaerobic work or to avoid condensation. A temperature probe is in contact with the cuvette to give the precise temperature of the reaction.

Diode array detectors are often used for cryo application as users try to get information about structure of reaction intermediates that can normally not be observed with standard stopped-flow instrumentation. In diode array mode spectra can be acquired at 0.8 ms interval. Fiber optics can also be connected to a photomultiplier tube for single wavelength measurements and in this case 10 μs resolution is achieved.

### Mixer and observation cell assembly

This part of the setup is immersed in the final cryosolvent. It contains a flow lines block that serves as reactant reservoir. These flow lines are made of inert material (PEEK) and are of sufficient volume so as to act as heat exchanger and allow equilibration of the reactants at the temperature of the cryosolvent before injection in the observation cuvette. In standard configuration volume of these lines are 200 μl but can be easily adapted. The mixer is fitted to the observation cell which can be any the standard stopped-flow cell ( 1 cm path offered in standard configuration).





The figure shows the reaction rate of alkaline hydrolysis of 2,4-dinitrophenylacetate (DNPA) by sodium methoxide. Solvent was methanol and the reaction rate was measured from 20 °C to -80 °C.

The Arrhenius plot obtained is given in the figure below and shows a remarkable linear slope over this 100 °C temperature range (activation energy was 4788 cal/mole).

#### CS -90 °C includes:

- Stopped-flow adaptor
- Umbilical link
- Mixing compartment including 1 cm light path cuvette
- 2 fiber optics ( 1.5 m long) with SMA connectors
- Isolast o'rings for full solvent compatibility
- Temperature probe
- Heater for temperature regulation.
- 20 liters cuve for cryosolvent
- coil for liquid N2 circulation

#### Not supplied

- Cryo-solvent ( isopropanol, ethanol...)
- Pressurized Liquid Nitrogen tank
- Tubing to connect cryo-stopped-flow to N2 tank

## Cryo-accessory for external cryostat

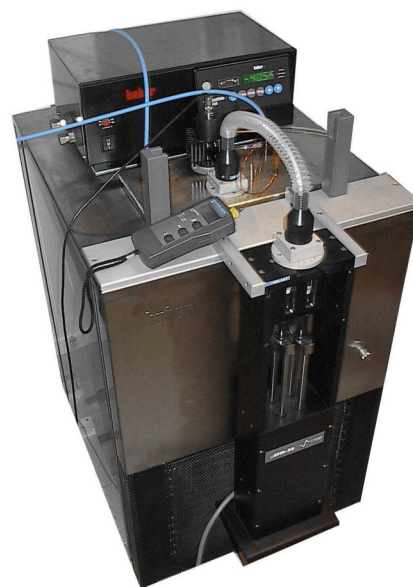
For users who prefer not handling liquid N2 for temperature control, Bio-logic also proposes a cryo-accessory compatible with commercial cryostat ( like models proposed by Huber). Several cryostat model can be proposed depending on the minimum temperature the user wants to reach. The mixing assembly and umbilic are similar to CS-90 °C. Stopped-flow is attached directly on the front of the cryostat (see picture) or on one side depending on models.

Temperature of the bath is set from the manual control unit and is stable within a 0.1C precision.

Customization of cryo-accessory is also possible to combine with existing cryostat or cryo-bath. The limitation is the aperture of the bath so the cryo accessory can fit into and the bath needs to be deep enough so all the mixing assembly is immersed in cryo-solvent. The minimum aperture should be 270 mm x 150 mm and minimal depth of the bath should be 150 mm.

#### Cryo-accessory includes:

- Stopped-flow adaptor
- Umbilical link
- Mixing compartment including 1 cm light path cuvette
- 2 fiber optics ( 1.5 m long) with SMA connectors
- Isolast o'rings for full solvent compatibility
- Temperature probe



SFM-20 attached to a Huber cryostat



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