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## 1) General terms

- Only non-pathogenic biological samples are accepted. Decree of the [July 18, 1994](#) establishing the list of biological pathogens, amended by Decrees of [April 17, 1997](#) and [June 30, 1998](#) (Decrees in French). The list of [biological pathogens](#) is available on the website of the IPBS.
- The platform is open to academics, which usually will be trained for data analysis. It's also open industrials and academics for carrying out studies and analysis by the platform, depending on the availability of the equipment and staff.
- Users can be trained to use of the equipment by themselves. Each applicant aiming to use the equipment must be trained, the mandatory training been renewed if not used during the past 6 months. The applicant has to follow and respect the instructions (part 2). In case of failure, the user can be excluded.
- The applicant has the responsibility of his samples, which will not be stored (unless explicit request).
- PAOL / OMNISEC is connecting to the IBS / EMBL internal network, to make easy printing and safeguarding the results. Generated data are safeguarded during 3 years. Past the delay, data will be deleted without notice.
- The platform doesn't guarantee the confidentiality of the data (unless explicit request).
- The user should acknowledge the platform in the publications: "This work used the platforms of the Grenoble Instruct-ERIC center (ISBG ; UAR 3518 CNRS-CEA-UGA-EMBL) within the Grenoble Partnership for Structural Biology (PSB), supported by FRISBI (ANR-10-INBS-0005-02) and GRAL, financed within the University Grenoble Alpes graduate school (Ecoles Universitaires de Recherche) CBH-EUR-GS (ANR-17-EURE-0003). We thank Caroline Mas and/or Aline Le Roy for assistance and/or access to the biophysics platform" and to communicate the reference of the article to the responsible of the platform.
- The personal of the platform is a co-author if he participates to the redaction of the article.

## Instructions for use

### Procedures

- Each applicant contacts the platform and fills the file "request of analysis". After discussion, the experimental conditions and the schedule are decided. The platform doesn't operate during the weekend.
- Commitments for samples, buffers, columns ... are described in part 3 and must be checked for each experiment.
- If something crops up, you must inform the local contact as soon as possible and fill an anomaly form.
- For each experiment, a PAOL / OMNISEC data acquisition form must be filled in:
  - Note the details of the experiment.
  - Note daily check values.
  - Note the anomaly, in case.

### Documents

- Protocols for PAOL / OMNISEC are available on the internal IBS network and in the ring binder nearby instruments.
- The reference manuals of the software can be consulted on instrument computer.

### Training

- The procedures described below are a part of those given during the **mandatory training**. Documentation is free available on the internal IBS network and in the ring binder nearby instruments.

## 2) Commitment on PAOL / OMNISEC

➤ The available columns are:

Instrument	Columns	Providers	Separation scale (kDa)	Composition and size of particules	Column dimension DxL (mm)	Buffer pH	Storage buffer
PAOL	PROTEIN KW-804	Shodex	until 1 000	Silice 7 µm	8.0 x 300	3 – 7.5	H <sub>2</sub> O, 0.3g/L NaN <sub>3</sub>
PAOL	PROTEIN KW-803	Shodex	until 700	Silice 5 µm	8.0 x 300	3 – 7.5	H <sub>2</sub> O, 0.3g/L NaN <sub>3</sub>
PAOL	PROTEIN KW-802.5	Shodex	until 150	Silice 5 µm	8.0 x 300	3 – 7.5	H <sub>2</sub> O, 0.3g/L NaN <sub>3</sub>
PAOL	PROTEIN KW-G	Shodex	Guard column		6.0 x 50		H <sub>2</sub> O, 0.3g/L NaN <sub>3</sub>
PAOL	WTC 050N5	Wyatt	15 - 5 000	Silice 5 µm	4.6 x 300		20% Ethanol
PAOL	WTC 050N5G	Wyatt	Guard column		6.0 x 50		20% Ethanol
PAOL / OMNISEC	Superdex 200 10/300 GL	GE Healthcare	10 – 600	cross-linked agarose and dextran 13 µm	10 x 300	3 – 12	20% Ethanol
PAOL / OMNISEC	Superose 6 10/300 GL	GE Healthcare	5 – 5000	cross-linked agarose 13 µm	10 x 300	3 – 12	20% Ethanol
PAOL / OMNISEC	Superdex 75 10/300 GL	GE Healthcare	3 – 70	cross-linked agarose and dextran 13 µm	10 x 300	3 – 12	20% Ethanol
OMNISEC	Superdex 30 10/300 GL	GE Healthcare	0.1 – 7	cross-linked agarose and dextran 9 µm	10 x 300	3 – 12	20% Ethanol

➤ The precautions of the columns are:

- A flow rate between 0.2 and 0.5 ml/min (typically: 0.5ml/min).
- Any changes in flow rate (e.g. stop the pump) must be done gradually.
- The silica columns are always preceded of a guard column.
- The elution buffer must:
  - o Have a pH adapted to the used column (see the table above).
  - o Contain at least 100 mM salt.
  - o Be filtered at 0.1 µm

In the case of a long experiment (few days), add, in the buffer, 0.3 g/L NaN<sub>3</sub> (life = 8 days)

**WARNING : NaN<sub>3</sub> = POISON => EPI !**

- A new silica column must be eluted during 1 week, with H<sub>2</sub>O Buffer (H<sub>2</sub>O, 0.3 g/L NaN<sub>3</sub>), at 0.5 ml/min.
- Equilibrate with the H<sub>2</sub>O Buffer all the columns, before and after each use (because possible reaction between alcohol and salts)
- The samples must be centrifuge during 10-15 min with the bench centrifuge at ~16800 xg, or filtered at 0.1µm.
- For membrane proteins, the elution buffer contains detergent: equilibrate the column with the elution buffer without detergent during few hours, then with detergent during 3 hours, before injecting the samples.
- General description of the columns according to providers.

**DATE AND RESPONSIBLE VISA: 9<sup>TH</sup> JUNE 2023, ALINE LE ROY AND CAROLINE MAS**