**Protein Identification and Quantification Service**

**(LC-MS/MS Analysis)**

**Requisition Form**

|  |
| --- |
| **Requested by** |
| **Name:**  |  |
| **Department/Organization:**  |  |
| **Address:**   |  |
| **Telephone:**  |  |
| **E-mail:**  |  |
| **Order date:** Order date. |  |

**Sample Information;**

|  |
| --- |
| **Sample Information - Please complete details on all pages & sign page 3**  |
| Source: (e.g. gel-band, freeze-dried or aqueous sample)  | Buffer composition for liquid or freeze -dried sample:  |
| Staining Method: (eg. Coomassie, silver\*, other) | Amount of protein in sample(s): ……………………………………….Purity of sample(s):……………………………………….Volume of liquid sample(s): ………………………………………. |
| Provide details for target database. | Chemicals used for reduction & alkylation, if any:  |
| No. of Samples (n):  |

**Number of sample:** XX

**Service method**

[ ]  In-solution digestion
[ ]  In-gel digestion (SDS-PAGE gel only)

**Note to Laboratory;** XXXXXXX

**Project Detail;**

**Client:** ………

**Services:** In-Gel Digestion

Sample Name: XXXX

**Sample Type:**Culture supernatant (*If other*, Please specify: )

**Organism:** Homo sapiens (*If other*, Please specify: )

**Order Date:** Order date.

**Report Date:** Report date.

**Note:** Click or tap here to enter text.

**Sample Information**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample No.** | **Sample Name** | **Sample Type** | **Note***If other*, Please specify |
|  |  | Sample type  |  |
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**Note;** If you have any question, please do not hesitate to contact proteomics service team

**Authorised Signature \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Lab use only:**

|  |  |
| --- | --- |
| **Prep Received:**  |  |
| **Plate No./Spot set:**  |  |
| **Processed/Operator:**  |  |
| **MS data analysis/Operator:**  |  |
| **QC No:**  |  |
| **MS data analysis/Operator:**  | **Username:** |  |
| **Password:** |  |
| **Enzyme Lot No:**  |  |
| **Report Reference:**  |  |
| **Special Considerations:**  |  |
| **Checked Report:**  |  |

**Guidelines for Sample Preparation**

**Gel band sample**

1. protect your samples from contamination with keratin. Ideally this should be done in a laminar flow hood
2. Excise gel bands on extremely clean surfaces using new razor blades or scalpels.
3. When excising bands of interest cut as closely to the staining boundary as possible.
4. Dice each gel slice into small pieces (~1 mm3) and place into 1.5 ml microcentrifuge tubes.
5. Add 200 μl or cover the gel pieces of 25 mM NH4HCO3

\*Pieces that are too large will result in reduced peptide recovery. Pieces that are too small can be lost during pipetting steps.

**Solution sample**

1. Must provide protein sample with 8M urea in 100 mM TEAB lysis buffer
2. Add 1x protease and/or phosphatase inhibitors to prevent degradation of extracted proteins
3. Measure protein concentration. Amount of protein should not less than 100 ug/sample

**Protocol of Cell lysis for protein extraction (Recommended)**

1. Add 100-300 ul or cover cells of lysis buffer (8M urea in 100 mM TEAB)
2. Add 1x protease and/or phosphatase inhibitors
3. Sonicate the lysate (pulse 10 sec., off 5 sec. for 5 min.)
4. Centrifuge at 13,000 g. for 5 minutes at 4°C.
5. Collect the supernatant into new 1.5 microcentrifuge tubes.
6. Determine protein concentration by the bicinchoninic acid method (BCA)