

Materials List for:**Purification of a High Molecular Mass Protein in *Streptococcus Mutans***Takatoshi Murata¹, Mamiko Yamashita¹, Masao Ishikawa^{1,2}, Koji Shibuya², Nobuhiro Hanada¹¹Department of Translational Research, Tsurumi University School of Dental Medicine²Laboratory for Oral Health ScienceCorrespondence to: Takatoshi Murata at murata-ta@tsurumi-u.ac.jpURL: <https://www.jove.com/video/59804>DOI: [doi:10.3791/59804](https://doi.org/10.3791/59804)**Materials**

| Name | Company | Catalog Number | Comments |
|-----------------------------------------------------|---------------------------------------|----------------|--------------------------------------------------|
| Agarose | Nippon Genetics | NE-AG02 | For agarose gel electrophoresis |
| Anaeropack | Mitsubishi Gas Chemical | A-03 | Anaerobic culture system |
| Anti-His-Tag monoclonal antibody | MBL | D291-7 | HRP-conjugated |
| BCA protein assay kit | Thermo Fisher Scientific | 23227 | Measurement of protein concentration |
| Brain heart infusion broth | Becton, Dickinson | 237500 | Bacterial culture medium |
| CBB R-250 | Wako | 031-17922 | For biofilm staining |
| Centrifugal ultrafiltration unit | Sartorius | VS2032 | Buffer replacement and protein concentration |
| Centrifuge | Kubota | 7780II | |
| Chromatographic column | Bio-Rad | 7321010 | For IMAC |
| Dialysis membrane clamp | Fisher brand | 21-153-100 | |
| Dialysis tubing | As One | 2-316-06 | |
| DNA polymerase | Takara | R045A | High-fidelity DNA polymerase |
| DNA sequencing | Eurofins Genomics | | |
| ECL substrate | Bio-Rad | 170-5060 | For western blotting |
| EDTA (0.5 M pH 8.0) | Wako | 311-90075 | Tris-EDTA buffer preparation |
| Electroporation cuvette | Bio-Rad | 1652086 | 0.2 cm gap |
| Electroporator | Bio-Rad | 1652100 | |
| EtBr solution | Nippon Gene | 315-90051 | For agarose gel electrophoresis |
| Gel band cutter | Nippon Genetics | FG-830 | |
| Gel extraction kit | Nippon Genetics | FG-91202 | DNA extraction from agarose gel |
| Imager | GE Healthcare | 29083461 | For SDS-PAGE and western blotting |
| Imidazole | Wako | 095-00015 | Binding buffer and elution buffer preparation |
| Incubator | Nippon Medical & Chemical Instruments | EZ-022 | Temperature setting: 4 °C |
| Incubator | Nippon Medical & Chemical Instruments | LH-100-RDS | Temperature setting: 37 °C |
| Membrane filter | Merck Millipore | JGWP04700 | 0.2 µm diameter |
| Microcentrifuge | Kubota | 3740 | |
| NaCl | Wako | 191-01665 | Preparation of binding buffer and elution buffer |
| NaH ₂ PO ₄ ·2H ₂ O | Wako | 192-02815 | Preparation of binding buffer and elution buffer |

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|-------------------------------------------------|--------------------------|----------------|--------------------------------------------------|
| NaOH | Wako | 198-13765 | Preparation of binding buffer and elution buffer |
| (NH ₄) ₂ SO ₄ | Wako | 015-06737 | Ammonium sulfate precipitation |
| Ni-charged resin | Bio-Rad | 1560133 | For IMAC |
| PCR primers | Eurofins Genomics | Custom-ordered | |
| Protein standard | Bio-Rad | 161-0381 | For SDS-PAGE and western blotting |
| Solvent filtration apparatus | As One | FH-1G | |
| Spectinomycin | Wako | 195-11531 | Antibiotics; use at 100 µg/mL |
| Sterile syringe filter | Merckmillipore | SLGV004SL | 0.22 µm diameter |
| <i>Streptococcus mutans</i> Δ <i>gtfC</i> | Stock strain in the lab. | | <i>gtfC</i> replaced with <i>spc'</i> |
| <i>Streptococcus mutans</i> UA159 | Stock strain in the lab. | | <i>S. mutans</i> ATCC 700610, Wild-type strain |
| Sucrose | Wako | 196-00015 | For biofilm development |
| TAE (50 ×) | Nippon Gene | 313-90035 | For agarose gel electrophoresis |
| Thermal cycler | Bio-Rad | PTC-200 | |
| Tris-HCl (1 M, pH 8.0) | Wako | 314-90065 | Tris-EDTA buffer preparation |