

SARS-CoV-2 Trimeric Spike Antigen - Best Handling Practices

Antigen Thawing	Thaw on ice in a fridge for min. 2 hours to reduce any heat stress on the antigen. Avoid multiple freeze thaw cycles.
Antigen Mixing	Mix thoroughly before use. The preferred method is with use of a roller mixer for min. 1 hour at 4°C. Avoid foaming of the sample by gentle rolling. Avoid vortexing.
Diluent Buffer Storage	Store diluent buffer at RT to prevent any glass cuvette condensation issues. Diluent buffer does not need to be mixed on a roller prior to Antigen dilution and OD280 measurements.
Diluent Buffer	LMNG diluent buffer supplied is identical to the buffer used in the Antigen formulation. Diluent buffer is supplied with all purified Antigen / LMNG preparations at a ml:mg ratio. E.g. 500 ml diluent buffer is supplied with 500 mg antigen. Optimal LMNG concentration is 0.001%. LMNG concentration higher than 0.05% interferes with OD280 measurements.
OD280 measurement	Use Molar Coefficient = 1.03 for protein concentration measurements.
OD280 measurement	Use a glass cuvette only.
OD280 measurement	Add diluent buffer to cuvette and zero the UV spec, dilute the sample in the cuvette directly, mix using a pipette 3 times. Use 1 in 10 dilution of Antigen in 1x dilution buffer to achieve an OD280 >0.1.
OD280 measurement	Polish glass cuvette between each OD280 measurement. Recommended protocol: Wash with MilliQ water several times Add 100 % Isopropanol several times Dry completely with compressed air to remove all isopropanol.
OD280 measurement	As the antigen is a sticky protein, polish glass cuvette at the end of each day. Recommended protocol: Add buffer to cuvette: 50 mM K ₂ HPO ₄ 10 mM EDTA 0.1 % Na Cholate, pH 7.2 Wash with MilliQ water several times Add 100 % Isopropanol several times Dry completely with compressed air to remove all isopropanol.
SDS-PAGE of Trimeric Antigen	Do not boil in sample buffer. Heat sample at 46°C for 30-45 mins only to prevent aggregation. Run a native page without SDS to confirm trimeric state of the Antigen.
Antigen Concentration	Use MWCO 100 kDa to limit detergent micelle concentration.