

Illumina Protein Prep at TGen: Sample Size and Handling Requirements

Table 1. Sample Size Requirements

Sample Type	Volume of Sample *
Serum	150 μ L
Plasma	150 μ L

*Lower volumes may be feasible in cases of limited availability, please enquire for assistance. Volume Listed is per replicate.

General sample processing requirements

Proper processing of the collected samples is **critical**. Many tubes have a minimum and maximum fill line, and these requirements should be followed, and no additional additives should be added to the samples. It is particularly important that time constraints are observed and that samples are not left at room temperature longer than necessary.

Samples should be fully processed and frozen at -80 °C within 2 hours of collection.

Final sample containers can be cryovials or 0.5mL matrix tubes. Please reach out to us if neither sample container is viable.

Note: Hemolyzed samples (pink to red in color) can confound true biomarker discovery. If available, send non-hemolyzed samples for your Protein Prep study. If only hemolyzed samples are available, please contact us.

Recommended general sample collection protocol

- Check the expiration date on all the tubes. If expired, replace with new ones.
- Perform venipuncture per institutional guidelines.
- If more than one sample type is collected, follow the collection order according to tube manufacturer's guidelines.

Plasma Processing

- Whole blood specimens for plasma samples must be collected in EDTA blood collection tubes. Do not freeze.
 - We cannot guarantee the performance of the Illumina Protein Prep protocol for samples collected using other blood collection tubes (for example, citrate plasma or heparin plasma).
- Begin processing whole blood specimens for plasma samples within 30 minutes of collection
- Plasma samples do not need to clot before placing on the centrifuge.
- Centrifuge collection tubes at room temperature. Centrifuge at $2200 \times g$ (RCF) for 15 minutes.
 - This speed optimizes removal of all cellular contents and platelets from samples.
 - Observe separation of blood cells and plasma, with plasma layer on top.
- Aliquot plasma samples **immediately** into labeled appropriate tube
 - Sample containers can be cryovials or 0.5mL matrix tubes. Please reach out to us if neither sample container is viable
- Aliquot only the plasma layer. To avoid disturbing the buffy coat, leave a residual of plasma and avoid the cell layer.
- Store aliquoted samples in a -80°C freezer. Storage effects at -20°C have not been evaluated for the Illumina Protein Prep assay.
 - Note: Plasma Samples do not need to clot, and should be centrifuged immediately after collection
- Avoid multiple freeze-thaw cycles of plasma samples.

Serum Processing

- Avoid adding anticoagulant to serum samples.
- Allow serum to clot for 60–90 min at room temperature before placing on the centrifuge.
- Centrifuge collection tubes at room temperature. Centrifuge at $2,200 \times g$ (RCF) for 15 minutes.
 - This speed optimizes removal of all cellular contents and platelets from samples.
 - Observe separation of blood cells and serum, with serum layer on top.
- Aliquot serum samples within 30 min of centrifugation into labeled appropriate tube
 - Sample containers can be cryovials or 0.5mL matrix tubes. Please reach out to us if neither sample container is viable

- Aliquot only the serum layer.
- Store aliquoted samples in a -80°C freezer. Storage effects at -20°C have not been evaluated for the Illumina Protein Prep assay.
- Avoid multiple freeze-thaw cycles of serum samples.