PROTIFI™
Bringing precision omics to life!™
https://protifi.com | info@protifi.com

S-Trap™ HYPERsol FFPE with PIXUL Megasonication Protocol

Tissue	Expected µg Soluble Protein/mg
Lymph Node	117
Heart	111
Pancreas	114
Colon	97
Spleen	96
Thyroid	93
Liver	84
Parathyroid	77
Cerebellum	77

Tissue	Expected µg Soluble Protein/mg
Cerebral Cortex	81
Kidney	74
Stomach	71
Uterus	60
Marrow	49
Testis	47
Prostate	43
Adrenal Gland	42
Lung	39

Protocol:

1. **Remove Excess Paraffin.** Trim FFPE blocks of excess paraffin using a sterile razor blade or scapula.
2. **Punch or Section.** Use a Biopsy Punch (for example a 1 mm Kai Miltex-Integra 33−31AA) to punch cores or alternatively section scrolls on a microtome. 5 mg of total FFPE tissue is recommended; pool as necessary.
3. **Solubilize and Decrosslink.**
    a. For tissue cores, dice them into small pieces with a scapula or razor blade then transfer to a 96-well plate. For scrolls, transfer to a 96-well plate; use tweezers to position on the bottom of the well.
    b. In the EU and Japan, completely remove paraffin from the sample with an organic solvent such as xylene or methyl tert-butyl ether (MTBE): add 100 µL, shake samples for 5 minutes at room temperature then remove all organic solvent to yield deparaffinized samples. Repeat as necessary to completely remove paraffin.
    c. Add 100 µL of S-Trap™ Solution 1, 2x Lysis Solution to 5 mg FFPE tissue or, if starting with less, add 20.0 µL per 1 mg.
    d. Allow samples to rehydrate overnight.
    e. Mega-sonicate at room temperature for 5 minutes on the PIXUL or until pieces have been dissolved: 50 N pulse at 1 kHz with a 20 Hz burst rate.
    f. Place on a heat block at 80 °C for 1 hour to reverse crosslinking. Make sure to poke small holes in the plate seal to relieve pressure and spin down condensate.
    g. Mega-sonicate for 6 minutes with the same settings as outlined above.
4. **Match Protein Loading.** Measure protein concentration with a BCA assay and match the concentration of protein in all samples by dilution as needed with S-Trap™ Solution 1, 2x Lysis Solution.
5. Process per standard S-Trap™ protocol.