

### Preparation of Tissue Homogenate

1. Collect spleen, lung, brain, kidney, liver, and heart tissues and treat with or without LPS (100 µg, i.p., 15 mins, 30 mins, 1 hr, 2 hrs, or 3 hrs).
2. Weigh tissue in a 2 mL microcentrifuge tube.
3. Add 500 µL of Cell Lysis Buffer (EPX-99999-000) per 100 mg of tissue.
4. Add one 5-mm Stainless Steel Bead, then assemble tubes into TissueLyser according to the manufacturer’s recommendations. We recommend to use 5-mm Stainless Steel Beads from Qiagen™ (Product No. 69989).
5. Homogenize tissue at 25 Hz for 0.5-3 mins as indicated in the table below.
6. Centrifuge the sample at 16,000 × g for 10 mins at 4°C.
7. Transfer the supernatant to a new microcentrifuge tube.
8. Measure the total protein concentration. We recommend using Bio-Rad™’s DC Protein Assay Kit.
9. Dilute samples to 10 mg protein/mL with 1X PBS.

To proceed with ProcartaPlex Protocol, add 25 µL of Universal Assay Buffer (EXP-11111-000) to 25 µL of the diluted sample to each sample well.

Tissue Lyser Condition			Total Protein Concentration (mg/mL)	
	Frequency (Hz)	Time (minutes)	Control Mouse	LPS-treated Mouse (2 hrs)
Spleen	25	0.5	114.1	115
Lung		0.5	108	96.3
Brain		0.5	92.8	70.5
Kidney		3	109.3	114.3
Liver		0.5	162	150.5
Heart		2	74.1	90.4

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#### Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code	Temperature limitation	Use by	Manufacturer	Consult instructions for use	Caution, consult accompanying documents
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Manufacturer’s address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.

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