# *UC Davis MMPC-Live Protocol*
# Preparation of Fecal Extracts for ELISAs

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## Summary:

This protocol is used to create a dispersion of feces or other gut contents in extract buffer to allow for the release of soluable analytes like lipocalin or calprotectin for subsequent analysis by ELISA or other assays.

## Reagents and Materials:

| *Reagent/Material* | *Vendor* | *Stock Number* |
|---|---|---|
| Walter Stern (100E) 4mm Glass Beads | Fisher Scientific | 11-312B |
| BioSpec Zirconia/Silica Beads (one pound bottle) 0.1mm diameter | Fisher Scientific | NC0362415 |
| 2 ml screwcap tubes | | |
| 1x PB | | |
| MPBio Fastprep-24 5G homogenizer, or equivalent | MP Biomedicals | 116005500 |
| Microcentrifuge capable of 12,000 x g | | |

## Protocol:

1. Prepare a 2 ml screwcap tube with ~3 mm deep 0.1mm dia. silica beads and one 4 mm dia. glass bead. This is roughly equivalent to the MPBio lysing matrix E, which would clearly also work for this assay.
2. Weigh prepared homogenization tube from step one.
3. Add 1-2 mouse fecal pellets (~8-50 mg wet weight) to a prepared homogenization tube.
4. Weigh homogenization tube with fecal material to determine fecal weight.
5. Add 500 µl Extract Buffer to each homogenization tube/feces sample.
6. Secure tubes and homogenize for 40 s at 6.0 M/s at room temperature in the MPBio Fastprep-24 5G homogenizer (human feces protocol).
7. Remove tubes and centrifuge for 5 min at >12,000xg at 4°C.
8. Transfer supernatant to a fresh microfuge tube and assay. Store remaining homogenate at -80°C.

## Reagent Preparation:

- **Extract Buffer**: 1x PBS + 0.1% Tween-20