CALCULATION EXAMPLES FOR BRADFORD AND ELISA

BRADFORD PROTEIN ASSAY

Protein concentration value:
1. After running the assay, you will have a value for a protein concentration per well of 0-5ug.
2. Calculate the ug protein per ul sample by dividing this number by the amount of sample placed in each well.
3. Multiply this number by the number of ul extracted (measured in graduated cylinder), resulting in a ug extracted value.
4. Divide this number by the g weight of soil, etc. used in the extraction. You now have a ug/g value that can be converted to mg/g by dividing by 1000.

Example:
OD reading = 0.386
ug/well concentration = 2.578ug/well
ul of sample/well = 5ul/well
amount of extract = 7200ul
weight extracted = 1.0 g

2.578ug/well ÷ 5ul/well x 7200ul ÷ 1.0g = 3712.32ug/g = 3.712mg/g

ELISA

Calculation to determine amount of sample needed per well:
Divide 0.2ug by the ug/ul value obtained from step 2 above. This gives the number of ul needed to yield a concentration of 0.2ug/well, a value in the middle of the curve. This allows you to use a single dilution for samples with a range of concentrations. We typically prepare 1:10 or 1:100 working dilutions of all samples to make for better pipetting volumes.

Example:
Bradford ug/well concentration = 2.578ug/well
ul of sample/well = 5ul/well

2.578ug/well ÷ 5ul/well = 0.5156ug/ul
0.2ug ÷ 0.5156ug/ul = 0.388ul/well
ul of 1:10 dilution needed = 3.9ul/well

For this sample 5ul of a 1:10 dilution would probably be used.
Protein concentration value:
1. After running the assay, you will have a value for a protein concentration per well of 0-0.4ug.
2. Calculate the ug protein per ul sample by dividing this number by the amount of sample placed in each well. Be sure to account for any dilutions made.
3. Multiply this number by the number of ul extracted (measured in graduated cylinder), resulting in a ug extracted value. Divide this number by the g weight of soil, etc. used in the extraction. You now have a ug/g value that can be converted to mg/g by dividing by 1000.

Example:
OD reading = 0.758
ug/well concentration = 0.21ug/well
ul of sample/well = 0.5ul/well
amount of extract = 7200ul
weight extracted = 1.0 g

0.21ug/well ÷ 0.5ul/well x 7200ul ÷ 1.0g = 3024ug/g = 3.024mg/g

% Immunoreactive (IR) protein:
The Bradford assay gives the total protein concentration, while ELISA gives the immunoreactive protein concentration. To calculate % immunoreactive protein, divide the ELISA value in mg/g by the Bradford value in mg/g, and multiply by 100%.

Example:
ELISA value = 3.024mg/g
Bradford value = 3.712mg/g

%IR = (3.024mg/g ÷ 3.712mg/g) x 100% = 81.5%