



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L605

Soil Enzyme Assays (Fluorometric Microplate Method)

Standard Operating Procedure

SOP Number	SOP-L605
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

FOR INTERNAL USE ONLY

This document is the property of the Environmental Analysis Program at Pomona College.
Unauthorized distribution is not permitted.

Table of contents

1 Purpose	2
2 Scope	2
3 Responsibilities	2
4 Definitions	2
5 Safety and Hazards	3
5.1 Required PPE	3
5.2 Emergency Procedures	3
6 Equipment and Materials	3
6.1 Equipment	3
6.2 Consumables	4
7 Target Enzymes and Substrates	4
8 Procedure	5
8.1 Soil Slurry Preparation	5
8.2 Substrate Stock Preparation	5
8.3 Microplate Layout	5
8.4 Incubation	5
8.5 Stopping and Reading	6
9 Data Analysis and Calculations	6
9.1 Activity Calculation	6
10 Quality Control	7
11 Waste Disposal	7
12 References	8
13 Related SOPs	8
14 Training Record	8

1. Purpose

This SOP describes the fluorometric microplate method for measuring the potential activity of extracellular enzymes in soils. Extracellular enzymes mediate the decomposition of organic matter and the cycling of carbon, nitrogen, phosphorus, and sulfur in soils. Assays use fluorogenic substrates based on 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (AMC), which release a fluorescent product upon enzymatic hydrolysis (Bell et al., 2013; German et al., 2011; Sinsabaugh & Moorhead, 1994). The method is high-throughput (96-well microplate format), sensitive, and widely used in soil biogeochemistry and ecosystem ecology.

2. Scope

- **Applies to:** Measurement of potential hydrolytic enzyme activities in field-moist, sieved (less than 2 mm) mineral and organic soils from EA Program sites, using MUB- and AMC-linked fluorogenic substrates in a 96-well microplate format
- **Does not apply to:** Oxidative enzyme assays (e.g., phenol oxidase, peroxidase), which use colorimetric substrates (L-DOPA); enzyme assays in aquatic samples (requires modified dilution); commercially available enzyme activity kits with different substrates
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Select target enzymes; approve substrate concentrations
Trained Personnel	Prepare reagents, run assays, analyze data
New Students	Complete training before independent use

4. Definitions

- **Fluorometric enzyme assay:** An enzyme activity assay in which substrate hydrolysis releases a fluorescent product (typically methylumbelliferone, MUB, or methylcoumarin, MCA) measurable at low concentrations by a fluorometer; more sensitive than colorimetric assays.
- **Maximum velocity (V_{max}):** The maximum rate of an enzyme-catalyzed reaction when substrate is saturating; expressed in units of nmol product g⁻¹ dry soil h⁻¹; reflects the potential activity of the enzyme in soil.
- **Methylumbelliferone (MUB):** A fluorescent product released by many hydrolytic enzymes when they cleave a methylumbelliferyl-linked substrate; detected at excitation ~365 nm and emission ~450 nm; the basis of most fluorometric soil enzyme assays.
- **Potential enzyme activity:** The enzyme activity measured under optimized laboratory conditions (excess substrate, controlled pH, temperature 25 °C); an index of the extant enzyme pool rather than actual in situ activity.

- **Quench correction:** A correction applied to fluorometric assays to account for reduction in fluorescence signal caused by soil colloids and dissolved organic matter; calculated from samples spiked with a known amount of MUB standard.
- **Substrate:** The molecule acted upon by an enzyme; in fluorometric assays, a synthetic substrate linked to MUB or MCA is cleaved by the target enzyme to release the fluorescent product.
- **Turnover rate:** The number of substrate molecules converted per enzyme molecule per unit time; an intrinsic property of the enzyme that depends on temperature, pH, and substrate concentration.

5. Safety and Hazards

Hazards

- **Chemical:** MUB and AMC substrates are dissolved in dimethyl sulfoxide (DMSO), which is readily absorbed through the skin and can carry dissolved solutes into the body. Sodium acetate buffer and sodium hydroxide (for pH adjustment) are mild to moderate irritants. Concentrated NaOH is corrosive.
- **Biological:** Soil samples may contain pathogens. Handle with gloves.
- **Physical:** Microplate reader UV lamp may cause eye strain with prolonged direct viewing.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves (double glove when handling DMSO solutions)

5.2. Emergency Procedures

For DMSO contact with skin, wash immediately with soap and water (do not use ethanol, which enhances absorption). For NaOH contact, flush with water for 15 minutes. Refer to [SOP-L002 Laboratory Safety](#).

6. Equipment and Materials

6.1. Equipment

Item	Notes
Fluorescence microplate reader	Excitation 365 nm, emission 450 nm
Multichannel micropipette (8-channel)	50–300 μL range
Single-channel micropipettes	10–100 μL and 100–1000 μL

Item	Notes
96-well black flat-bottom microplates	Non-treated; opaque walls to prevent bleedthrough
Orbital shaker or plate shaker	
Analytical balance	For weighing soil
pH meter	For buffer preparation
Centrifuge (optional)	For clarifying soil slurries

6.2. Consumables

Item	Notes
4-Methylumbelliferyl substrates	See enzyme table (Section 6)
7-Amino-4-methylcoumarin substrates	For leucine aminopeptidase
4-Methylumbelliferone (MUB) standard	For calibration curves
7-Amino-4-methylcoumarin (AMC) standard	For calibration curves
DMSO (ACS grade)	Solvent for substrate stocks
Sodium acetate buffer (50 mM, pH 5.0)	Or Tris buffer pH 7.8 for alkaline soils
NaOH (1 N)	To stop reactions and enhance fluorescence
DI water (Type II)	
Reagent reservoirs	For multichannel pipetting
Adhesive plate seals	For incubation

7. Target Enzymes and Substrates

The following enzymes are routinely assayed in the EA Program. Other MUB- or AMC-linked substrates can be added using the same general protocol.

Table 1: Target enzymes, substrates, and associated nutrient cycles.

Enzyme	Abbreviation	Substrate	Nutrient Cycle	Substrate Conc.
β -1,4-glucosidase	BG	4-MUB- β -D-glucopyranoside	Carbon	200 μ M
Cellobiohydrolase	CBH	4-MUB- β -D-cellobioside	Carbon	200 μ M
β -1,4-N-acetylglucosaminidase	NAG	4-MUB-N-acetyl- β -D-glucosaminide	Carbon / Nitrogen	200 μ M
Acid (alkaline) phosphatase	AP	4-MUB-phosphate	Phosphorus	200 μ M
β -1,4-xylosidase	BX	4-MUB- β -D-xylopyranoside	Carbon	200 μ M
Leucine aminopeptidase	LAP	L-Leucine-7-AMC	Nitrogen	200 μ M
Sulfatase	SUL	4-MUB-sulfate	Sulfur	200 μ M

8. Procedure

8.1. Soil Slurry Preparation

1. Weigh 1.0 g of field-moist, sieved (less than 2 mm) soil into a clean container.
2. Add 125 mL of 50 mM sodium acetate buffer (pH adjusted to match the soil pH, typically 5.0 for acidic soils or 7.8 for alkaline soils) (German et al., 2011).
3. Homogenize with a magnetic stir bar or by vortexing for 1 minute.
4. Keep the slurry stirring continuously during pipetting to maintain a uniform suspension.

8.2. Substrate Stock Preparation

1. Dissolve each MUB or AMC substrate in DMSO to prepare a 10 mM stock solution. Store at -20°C in amber vials; stable for at least 6 months.
2. Prepare working solutions by diluting the DMSO stock in buffer to the target concentration (typically 200 μM final concentration in the well).
3. Prepare MUB and AMC calibration standards in buffer at concentrations of 0, 0.5, 1, 2.5, 5, 10, and 25 μM .

8.3. Microplate Layout

For each soil sample, set up the following wells (8 analytical replicates per enzyme) (Bell et al., 2013):

Well Type	Contents	Purpose
Sample assay (n = 8)	200 μL slurry + 50 μL substrate	Enzyme activity
Sample control (n = 8)	200 μL slurry + 50 μL buffer	Soil background fluorescence
Substrate control (n = 8)	200 μL buffer + 50 μL substrate	Substrate autofluorescence
MUB/AMC standard curve	200 μL buffer + 50 μL standard	Calibration
Blank	200 μL buffer + 50 μL buffer	Instrument blank

8.4. Incubation

1. Seal the plate with an adhesive seal.
2. Incubate at 20°C (or field-relevant temperature) in the dark on a plate shaker at low speed for 2–4 hours. The incubation time depends on expected activity; 3 hours is a standard starting point (Sinsabaugh & Moorhead, 1994).

 Tip

For soils with unknown activity, run a pilot plate at 1, 2, and 4 hours to identify the linear range. Activity calculations assume linearity over the incubation period; if the reaction plateaus, shorten the incubation.

8.5. Stopping and Reading

1. After incubation, add 10 μL of 1 N NaOH to each well to stop the reaction and maximize fluorescence of the MUB/AMC product (the fluorophore is brightest above pH 10).
2. Read fluorescence on the microplate reader at excitation 365 nm / emission 450 nm within 5 minutes of adding NaOH.
3. Export the raw fluorescence data for analysis.

9. Data Analysis and Calculations

9.1. Activity Calculation

For each enzyme, calculate the net fluorescence:

$$F_{\text{net}} = F_{\text{sample}} - F_{\text{soil control}} - F_{\text{substrate control}} + F_{\text{blank}}$$

Convert net fluorescence to nmol of product released using the MUB or AMC standard curve (linear regression of fluorescence vs. concentration).

Calculate enzyme activity:

$$\text{Activity (nmol g}^{-1}\text{h}^{-1}) = \frac{F_{\text{net}}/m \times V_{\text{well}}}{M_{\text{soil}} \times t}$$

where m = slope of the standard curve (fluorescence per nmol), V_{well} = total well volume (L), M_{soil} = dry mass equivalent of soil in the well (g, corrected for moisture content per [SOP-L400](#)), and t = incubation time (h).

```
# Example enzyme activity calculation
library(tidyverse)

# Standard curve data
standards <- tibble(
  conc_uM = c(0, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0),
  fluorescence = c(120, 850, 1580, 3750, 7200, 14100, 34500)
)

# Fit linear model
cal_model <- lm(fluorescence ~ conc_uM, data = standards)
```

```
slope <- coef(cal_model)[2] # fluorescence units per uM

# Sample data (means of 8 replicates)
F_sample <- 5200
F_soil_ctrl <- 350
F_sub_ctrl <- 180
F_blank <- 125

F_net <- F_sample - F_soil_ctrl - F_sub_ctrl + F_blank

# Convert to concentration (uM in well)
conc_well <- F_net / slope

# Calculate activity
V_well_L <- 250e-6 # 250 uL total well volume in liters
soil_fresh_g <- 1.0 # g fresh soil in 125 mL slurry
frac_in_well <- 200 / (125 * 1000) # fraction of slurry in well
soil_in_well_g <- soil_fresh_g * frac_in_well
moisture <- 0.25 # gravimetric moisture content
soil_dry_g <- soil_in_well_g * (1 - moisture)
incubation_h <- 3

activity <- (conc_well * V_well_L * 1e6) / (soil_dry_g * incubation_h)
# Result in nmol / g dry soil / h

cat(sprintf("Net fluorescence: %.0f\n", F_net))
cat(sprintf("Activity: %.1f nmol g-1 h-1\n", activity))
```

10. Quality Control

- Run 8 analytical replicates per enzyme per sample. Report the mean and coefficient of variation; CV should be less than 20%.
- Include substrate and soil quench controls on every plate.
- Run the MUB/AMC standard curve on each plate (do not carry over calibrations between plates, as reader sensitivity may drift).
- Include a laboratory reference soil in each batch to track inter-batch variability.
- Verify that activity is linear over the incubation period by running a time-course pilot for new soil types.

11. Waste Disposal

Waste Type	Disposal Method
Plate contents (soil slurry + substrates)	Drain with copious water (dilute, non-hazardous at assay concentrations)
DMSO substrate stock waste	Collect in organic waste; EH&S pickup
NaOH waste	Neutralize and drain
Used microplates	Standard trash
Used pipette tips	Standard trash

12. References

- Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D. (2013). High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *Journal of Visualized Experiments*, (81), e50961. <https://doi.org/10.3791/50961>
- German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry*, 43(7), 1387–1397. <https://doi.org/10.1016/j.soilbio.2011.03.017>
- Sinsabaugh, R. L., & Moorhead, D. L. (1994). Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. *Soil Biology and Biochemistry*, 26(10), 1305–1311. [https://doi.org/10.1016/0038-0717\(94\)90211-9](https://doi.org/10.1016/0038-0717(94)90211-9)

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L400	Soil Moisture (Gravimetric)	Moisture correction for dry-mass calculations
L409	Microbial Biomass Carbon	Complementary microbial activity measure
L408	Solvita CO ₂ Burst	Related microbial activity metric
F400	Soil Sampling	Upstream: sample collection

14. Training Record

Before performing soil enzyme assays independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Review the SDS for DMSO, NaOH, and the fluorogenic substrates
4. Observe a trained operator perform a complete assay (slurry preparation through plate reading and data export)
5. Perform the assay under supervision with acceptable QC results (replicate CV less than 20%; standard curve $r^2 > 0.99$)

6. Demonstrate correct use of the multichannel pipette and microplate reader
7. Be signed off by the PI or lab manager