May 29, 2025

# Measuring Glycine Betaine concentrations in *Typha/Spartina*

DOI
https://dx.doi.org/10.17504/protocols.io.q26g75rr9lwz/v1

Suzanne M Thomas[1], Zoe Cardon[1], Ilana Stein[1]
[1]Marine Biological Laboratory

Suzanne M Thomas
Marine Biological Laboratory





**DOI:** https://dx.doi.org/10.17504/protocols.io.q26g75rr9lwz/v1

**Protocol Citation:** Suzanne M Thomas, Zoe Cardon, Ilana Stein 2025. Measuring Glycine Betaine concentrations in Typha/Spartina. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g75rr9lwz/v1



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** March 25, 2025

**Last Modified:** May 29, 2025

**Protocol Integer ID:** 125031

**Keywords:** saltmarsh, glycine betaine, plant stress, sediment, glycine betaine content, spartina gycine betaine, measuring glycine, betaine concentrations in typha, microbes in salt marsh, betaine concentration, roots of typha angustifolia, salinity, many tidal marsh plant species, salt marsh, important aspect of salt tolerance, different salinity, typha angustifolia, compatible osmolyte that many plant, salt tolerance, marine sediment, compatible osmolyte, glycine betaine concentrations in typha, measuring glycine betaine concentration

**Funders Acknowledgements:**
**Simons Foundation**
Grant ID: 1247132
**DOE**
Grant ID: DE-SC002470

## Abstract

Gycine betaine (GB) is a compatible osmolyte that many plants produce in response to various types of stress, particularly drought and salinity (Ashraf & Foolad, 2007; Jarin *et al.*, 2024). GB accumulation is an important aspect of salt tolerance for many tidal marsh plant species (Adrian-Romero *et al.*, 1998), as well as being a substrate for methanogenesis by microbes in salt marsh and marine sediments (Boysen *et al.*, 2022; Bueno De Mesquita *et al*). We measured glycine betaine content in both the leaves and roots of *Typha angustifolia* grown under different salinities, using the method of Grieve and Grattan (1983), as modified by Valdez-Bustos et al (2016). We made a further modification, using a plate reader instead of a spectrophotometer, which makes it possible to analyze samples more quickly.

## Image Attribution

All images taken by Suzanne Thomas

## Materials

- Betaine, anhydrous **Spectrum Chemical MFG Corp Catalog #**B1572
- Sulfuric acid **Fisher Scientific Catalog #**A300C-212
- 1,2-dichloroethane **Thermo Fisher Scientific Catalog #**032462.K2
- potassium iodide **Thermo Fisher Scientific Catalog #**A12704.18
- Iodine flakes **Fisher Scientific Catalog #**137-100

Equipment:

**Equipment**

| | |
|---|---|
| MM200 | NAME |
| Grinding mill | TYPE |
| Retsch | BRAND |
| MM200 | SKU |

Equipment

**Synergy H1** NAME

Plate Reader TYPE

BioTek BRAND

H1M SKU

Equipment

**96 well plate** NAME

acrylic UV transparent plate TYPE

Corning BRAND

3635 SKU

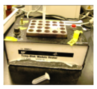
Equipment

**block heater** NAME

module block heater TYPE

American Scientific BRAND

2025-1 SKU



Equipment

**Neoprene** NAME

lab gloves TYPE

MicroFlex BRAND

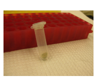
NPG-888 SKU

Equipment

| | |
|---|---|
| **Eppendorf 5415R Refrigerated Centrifuge** | NAME |
| Refrigerated Centrifuge | TYPE |
| Eppendorf | BRAND |
| EP-5415R | SKU |

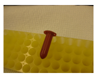
Equipment

| | |
|---|---|
| **Eppendorf tubes** | NAME |
| 2mL clear | TYPE |
| USA Scientifc | BRAND |
| 1620-2700 | SKU |



Equipment

| | |
|---|---|
| Eppendorf tubes | NAME |
| 2mL amber | TYPE |
| Eppendorf | BRAND |
| 022363379 | SKU |



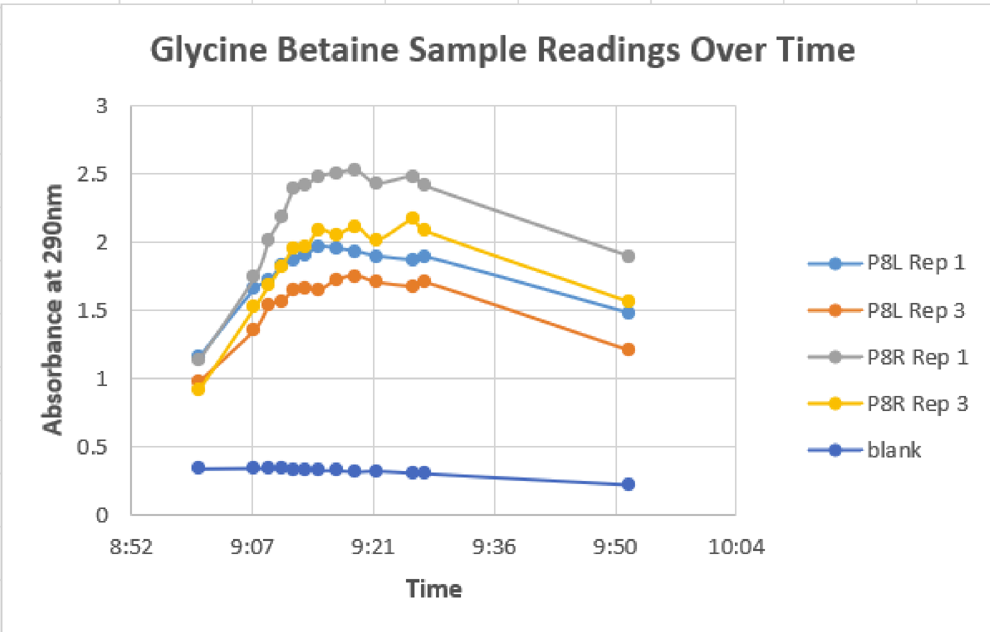
## Troubleshooting

## Safety warnings

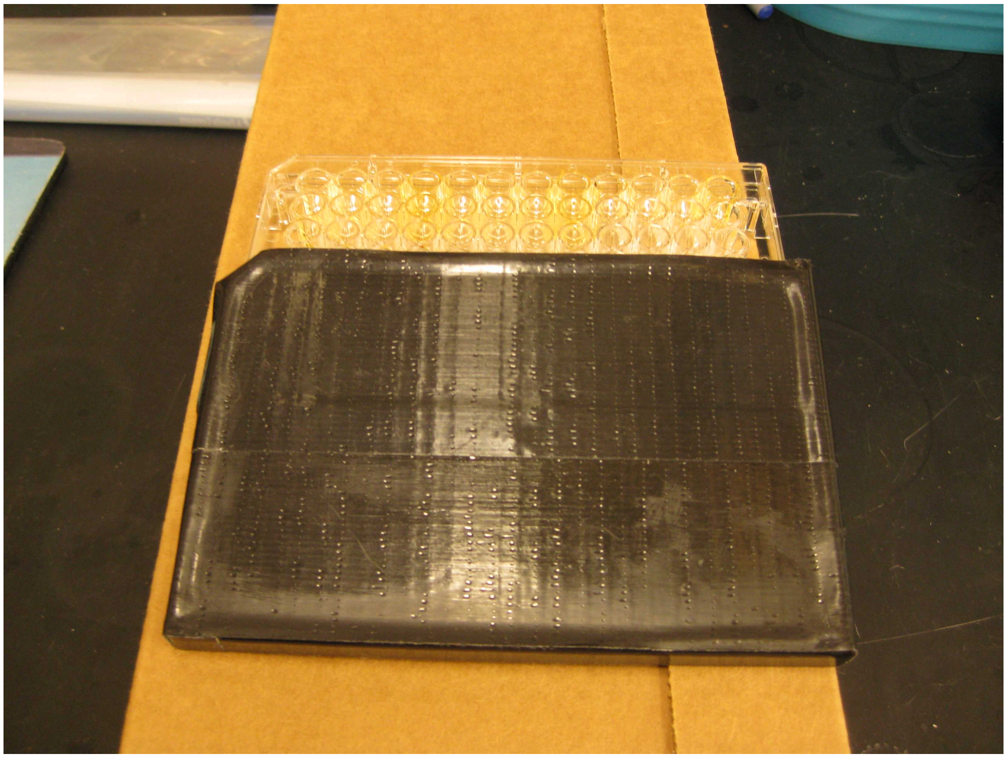
1,2-dichloroethane (also known as ethylene dichloride) is toxic, corrosive, and carcinogenic. It is also highly volatile. This method calls for using undiluted 1,2-dichloroethane. It is imperative to use neoprene gloves, safety glasses, lab coat, and work in a fume hood at all times.

## Before start

Iodine is light sensitive. Excess iodine will be removed in Step #20, but iodine will also be incorporated into the betaine-iodide crystals. All steps where the iodine reagent is involved (Steps 14-27) were done under low light. The graph below shows how quickly iodine reacts with light. A black cover was used to protect the samples from light as they were being pipetted into the 96 well plate to read the absorbance.




Repeated absorbance readings on samples (plate removed from plate reader between readings- volatile dichlorethane could damage the plate reader). The iodine reacts with light and quickly changes the absorbance.



96 well plate lid covered with black duct tape.  This helps to decrease the reaction of iodine with light while samples are being pipetted to read absorbance in the plate reader.

It is critical to remove the iodine reagent when it is in excess.  In the photo below, I ran samples in quadruplicate, intentionally leaving a small drop of iodine solution in the vial with the betaine iodide crystals.  This shows that it is critical to remove the iodine reagent in Step #20.

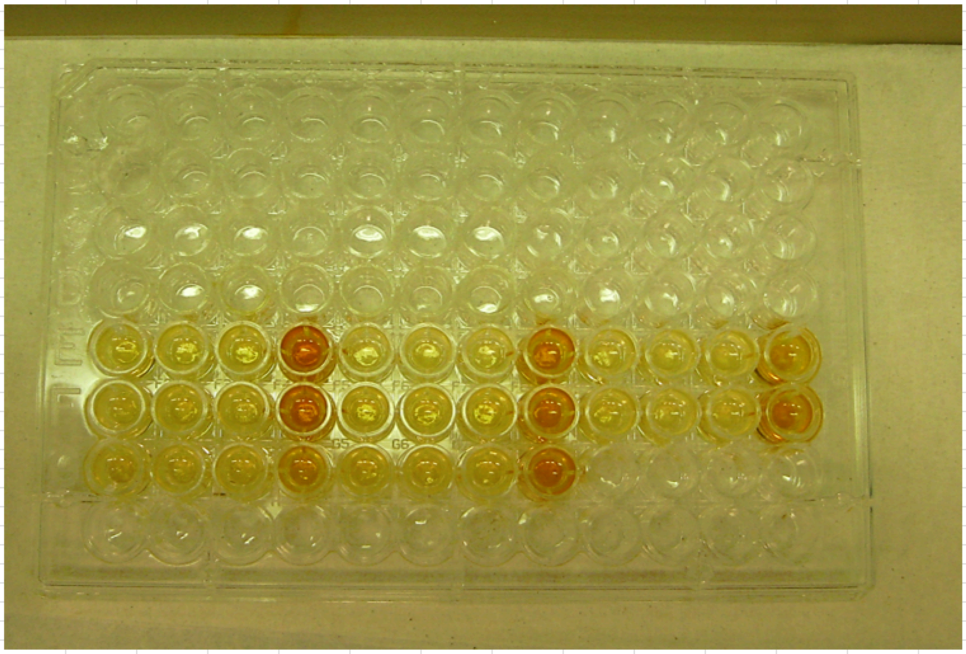


Photo of 96 well plate.
For the 4th rep of each set, I intentionally left one small drop of iodine solution to see what effect that would have on the final value.

96 well plate showing samples run in quadruplicate, where the 4th sample of each set was intentionally contaminated with a small drop of iodine solution from Step # 20.

## Collect and prepare plant material

12h 10m

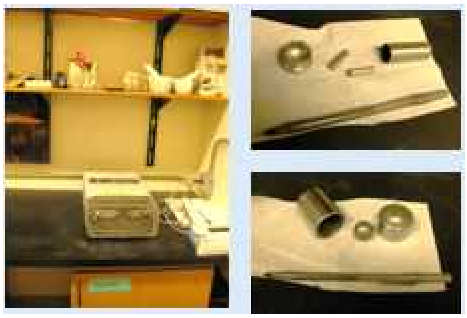
1 snip ~20cm of a leaf (we are currently looking at glycine betaine in saltmarsh and brackish marsh plants (*Sporobolus* and *Typha*)) and place in a labeled paper bag

2 Place sample + bag in a 60°C drying oven overnight or longer

12h

3 Grind sample into a homogenized powder

10m



Retsch mill grinder and stainless steel grinding vessels

4 Store ground sample in a closed vial

## Make Reagents and Standards

4h 45m

5 **2N Sulfuric Acid**

Add 28 mL [M] 36 Molarity (M) H2SO4 (sulfuric acid) to ~300mL deionized water in a 500mL volumetric flask. Swirl to mix. When cool, fill volumetric flask to 500mL. Invert to mix 10x. Transfer to a glass bottle for storage.

1h

**Safety information**

- Work in a fume hood when handling full-strength sulfuric acid.
- Use nitrile or latex gloves and remove gloves immediately if you come into contact with any concentrated acid.
- Wear eye protection.
- Wear a lab coat.

6 **Potassium Iodide - Iodine Solution** 2h

1.57 g I2 (iodine flakes/chunks) + 2 g KI (potassium iodide) + 10 mL deionized water in a dark bottle. Swirl from time to time to mix. It will take some time for the KI-I2 to dissolve.

Safety information

Use appropriate PPE when handling these chemicals- lab coat, safety glasses, latex or nitrile gloves.

7 **Prepare 1,2-dichloroethane (also known as ethylene dichloride)** 15m

Pour 1,2-dichloroethane into a smaller glass bottle (50 or 100mL) as a working reagent (full strength)

Safety information

**This chemical is very hazardous! It is toxic, corrosive, carcinogenic, and flammable.**
You must:

- Work in a fume hood
- Wear special lab gloves- **neoprene**
- Wear safety glasses and a lab coat
- Always use and store this chemical in a fume hood. It is very volatile

8 **Make Standard Stock Solution (200mg/mL)** 1h 30m

4 g betaine + 20 mL 2N H2SO4

From this solution, I made standards of 0, 1, 2, 5, 10, 20, 50, 100, 200, and 500 µg/mL

## Extract Glycine Betaine from Ground Plant Material (timing estimates for ~15 samples) 2d 19h 45m

9 Weigh out ~ 0.02 g ground material and place in a 2mL Eppendorf tube (can be clear) 30m

10 Add 1.5mL 2N H2SO4 to tube with ground plant material 10m

Safety information

You should be wearing gloves and safety glasses

11 Vortex closed tube to mix

12 Heat samples to 60 °C for 10 minutes in a heating block (or similar) 10m

13 Centrifuge sample tubes at ~ 14,000 rpm, 22°C (our centrifuge only goes to 13,200rpm and that has been fine) for 10-25 minutes at room temperature. The centrifugation time depends on how much plant material is in the vial and how dense it is. 25m

14 Transfer 125 µL sample supernatant to an amber 2mL Eppendorf tube and add 50 µL cold KI-I2 solution 20m

Note

The iodine solution is said to be light sensitive, so I do this step with the overhead lab lights off and a small light on to see by.

15 Cap tube and place mixture on ice immediately. Ice should be kept in a cooler with a lid to keep the samples dark.

16 Place cooler with ice and samples in a refrigerator overnight. Samples should remain at 0-4°C for 16 hrs. 16h

17 Cool Centrifuge to 0°C. Ours take 30-60 minutes to cool down. 1h

Note

**It is crucial that everything stays cold during these next steps! The betaine iodide complexes that form can dissolve at temps > 0°C.**

18 Centrifuge samples at 0°C and ~ 14,000 rpm for 30 minutes. 30m

19 Remove samples from centrifuge and return to ice. 5m

20 Remove supernatant from sample tube. Only the crystals of betaine iodide should remain. Do a quick visual inspection to ensure that all liquid iodine reagent has been removed. Supernatant should be disposed of in a labeled hazardous waste container. 15m

Note

Again working with the overhead lab lights off and a small light on to see by.

21 Once the supernatant has been removed, the sample tube may be placed in a holder at room temperature.

22 Move samples to a fume hood and change gloves to neoprene gloves. Double check that you have your safety glasses on. 10m

Note

Continue to work in low light, but dichloroethane is very nasty, so we want to make sure we can see well. Our lab has two banks of lights. We work with our fume hood light off, but with one bank of lab lights on.

23 Add 1.4 mL 1,2- dichloroethane to sample vial with crystals. Cap. 10m

Safety information

Collect pipette tips in a glass beaker to evaporate in the fume hood.  Then dispose of as hazardous waste when dry.

24 Let crystals dissolve for 48 hrs.  I agitate the vial holder several times over this period to encourage dissolution.  We place an opaque plastic box over the samples to ensure darkness.

2d

Safety information

These vials need to stay in the fume hood for this step!  The dichloroethane is very volatile.

## Determine Concentration of Glycine Betaine in Samples on a Plate Reader

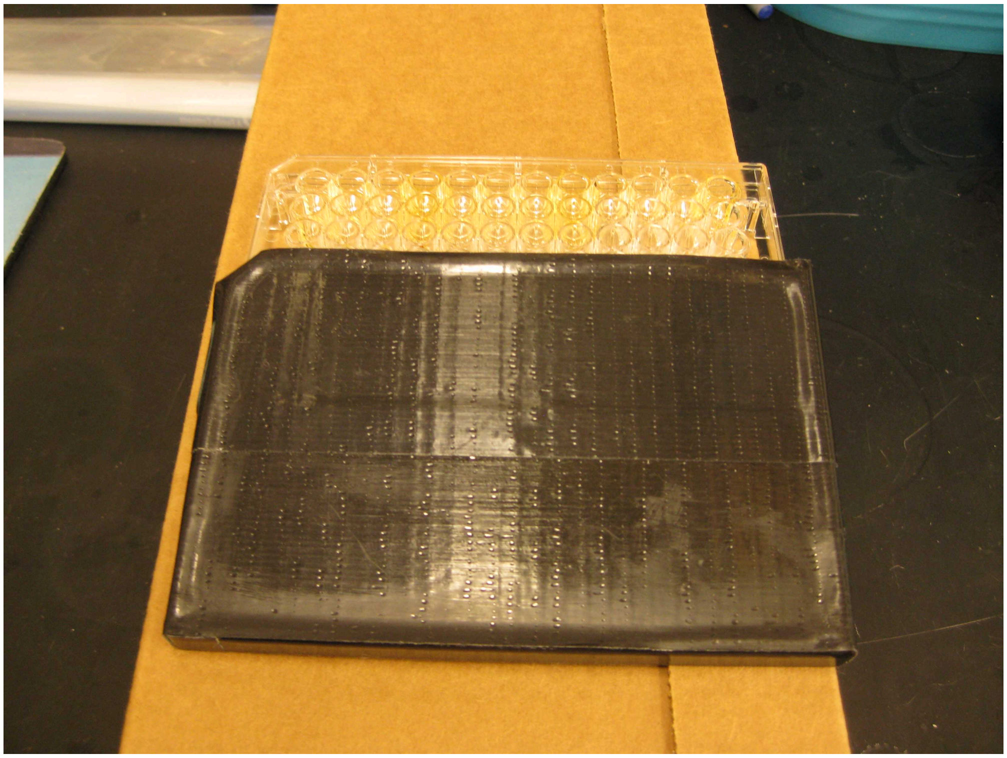
1d 0h 22m 30s

25 Agitate samples in holder to mix.  Prepare plate reader and 96 well plate.  We have found that reading one row of 12 samples at a time works well for us, as long as each well in the row is covered as it is filled.  The plate reader should be set to read absorbance at 290nm (and 365nm, although we're not sure this wavelength is necessary).

10m

Note

This step can also be done on a spectrophotometer in a fume hood.  You will have to use a removable glass/quartz cuvette (as opposed to pump tubing and a sipper unit. The dichlorethane would dissolve pump tubing).  **Being in a fume hood is critical to working with 1,2-dichloroethane.**



96 well plate lid covered with black duct tape. When 100µL of sample is pipetted into a well, the plate cover is repositioned to cover the well and block out most of the light. The 96 well plate itself is clear, so some light gets in through the bottom plate.

Note

I am again working with only half the lab lights on and am in the fume hood. It is crucial to cover each well after it is filled with sample. The iodine that is released from the betaine iodide crystal is very light sensitive. I use a 96 well plate lid covered in black duct tape to cover each well as I fill a row. Then I place the covered 96 well plate on the plate reader arm and remove the cover as the plate is being pulled into the plate reader.

26 Add 100µL of sample to a well and read a row of 12 samples at a time in the plate reader at 290nm

2m 30s

Safety information

**Working in a fume hood**, move quickly (light sensitive extract) but carefully with these samples. The dichloroethane is toxic, corrosive, carcinogenic, and volatile. Do NOT leave the plate in the plate reader for any longer than is necessary. The fumes will degrade the instrument.
All pipette tips should be ejected into a glass beaker. Once dry (overnight), dispose of as hazardous waste.

27 Repeat until all samples have been read. 10m

28 Allow samples to evaporate from the wells. Yes, you are still in a fume hood! The well will have been distorted and stained from the sample. The plates are single-use for this experiment. 1d

## Protocol references

Adrian-Romero M, Wilson SJ, Blunden G, Yang M-H, Carabot-Cuervo A, Bashir AK. (1998). Betaines in coastal plants. *Biochemical Systematics and Ecology* 26: 535–543.
https://doi.org/10.1016/S0305-1978(98)00013-1

**Ashraf M, Foolad MR. 2007.** Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* **59**: 206–216.
https://doi.org/10.1016/j.envexpbot.2005.12.006

**Boysen AK, Durham BP, Kumler W, Key RS, Heal KR, Carlson LT, Groussman RD, Armbrust EV, Ingalls AE**. **2022**. Glycine betaine uptake and metabolism in marine microbial communities. *Environmental Microbiology* **24**: 2380–2403.
https://doi.org/10.1111/1462-2920.16020

**Bueno De Mesquita CP, Wu D, Tringe SG**. **2023**. Methyl-Based Methanogenesis: an Ecological and Genomic Review. *Microbiology and Molecular Biology Reviews* **87**: e00024-22.
https://doi.org/10.1128/mmbr.00024-22

Citation

C.M. Grieve and S.R. Grattan (1983)
. Rapid Assay for determination of water soluble quaternary ammonium compounds. Plant and Soil.

https://doi.org/10.1007/BF02374789 LINK

**Jarin A, Ghosh UK, Hossain MdS, Mahmud A, Khan MdAR**. **2024**. Glycine betaine in plant responses and tolerance to abiotic stresses. *Discover Agriculture* **2**: 127.
https://doi.org/10.1007/s44279-024-00152-w

Citation

Valadez-Bustos MG, Aguado-Santacruz GA, Tiessen-Favier A, Robledo-Paz A, Muñoz-Orozco A, Rascón-Cruz Q, Santacruz-Varela A
(2016)
. A reliable method for spectrophotometric determination of glycine betaine in cell suspension and other systems.

https://doi.org/10.1016/j.ab.2015.12.015 LINK

Citation

Noreen Akhtar, Mansoor Hameed, Fahim Nawaz, Khawaja Shafique Ahmad, Abdul Hamid, Claudia Segovia-Salcedo, Muhammad Muslim Shahnaz
(2017). Leaf anatomical and biochemical adaptations in Typha domingensis Pers. ecotypes for salinity tolerance.
Botanical Sciences.

DOI: 10.17129/botsci.886 LINK