

Calculation of PGal DH Specific Activity

For convenience, the specific activity calculations are made on a mass basis.¹ A contrived example will be used to demonstrate how this calculation is made.

Step 1: Calculation of fresh-mass equivalent in extract.

Suppose 50 mg of fresh leaf is homogenized in 1 ml extraction cocktail. Assume fresh leaflet is 85% water. Then, the total aqueous phase of the extract is $1.000 \text{ ml} + (0.85 \times 0.05 \text{ g}) = 1.042 \text{ ml}$. Thus, each microliter of the soluble extract phase would be equivalent to $48 \times 10^{-9} \text{ kg}$ fresh leaf; i.e., $(50 \times 10^{-6} \text{ kg}) / 1042 \mu\text{l}$. Or, 30 μl extract is equivalent to $1.44 \times 10^{-6} \text{ kg}$ leaf.

Step 2: Calculation of substrate reacted per unit time.

Assume:

	A ₃₄₀ (5 minutes)	A ₃₄₀ (15 minutes)	A ₃₄₀ /minute
Complete reagent	0.80	0.51	0.029
Complete reagent - PGA	0.82	0.77	0.005
Complete reagent - extract	0.74	0.74	0.000

Thus--after subtraction of the blank rate-- the extract-dependent, substrate-dependent decline in A₃₄₀ is 0.024 O.D./minute. The millimolar extinction coefficient of NADPH (1 cm light path) is 6.270 at 340 nm. Thus, a solution of 0.16 mM (= 1/6.27) has an A₃₄₀ of 1 O.D. It follows that a $\Delta A_{340} / \Delta t$ ime of 0.024 O.D./minute = 3.84 $\mu\text{molar}/\text{minute}$ [= $(0.16 \text{ mM/O.D.}) \times (0.024 \text{ O.D./minute})$].

¹In practice, you will not weigh the leaf samples that you extract. You will take punches and extract immediately. (It is important to extract as quickly as possible, to minimize the changes in physiological state of the tissue.) Assume that each leaf punch weighs 50 mg (fresh mass).

In a total volume of 3.06 ml (= 3 ml cocktail + 0.03 ml extract + 0.03 ml PGA), the substrate consumed is 11.8 nmol/minute [= $(3.06 \times 10^{-3} \text{ liter}) \times (3.84 \times 10^{-6} \text{ mol/liter}/\text{minute})$].

Step 3: Calculation of specific activity.

Specific activity is defined as substrate reacted·time⁻¹·tissue⁻¹. Thus, the specific activity = $11.8 \text{ nmol}\cdot\text{minute}^{-1}\cdot(1.44 \times 10^{-6} \text{ kg})^{-1}$ □ $8.2 \text{ mmol}\cdot\text{minute}^{-1}\cdot\text{kg}^{-1}$.

Important: Follow through with all units in your calculations.