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Publisher’s note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.
Protocol to measure β-galactosidase in Drosophila extracts using a CPRG assay

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SUMMARY

The quantification of β-galactosidase activity is routinely required by laboratories worldwide. We present a cost-effective, highly replicable, simple technique for quantifying β-galactosidase-specific activity from crude extracts made from whole organisms or dissected tissues or cells. Extracts are prepared and measured without the need for any specialized equipment, and tissue is ground manually by pestle and measured by colorimetric CPRG and Bradford assays. This protocol describes the assay using Drosophila extracts but could be applied to any biological system of interest. For complete details on the use and execution of this protocol, please refer to Seroude et al. (2002),¹ Poirier et al. (2008),² and Barwell et al. (2017).³

BEFORE YOU BEGIN

Since the breakthrough discovery of the lac operon by Jacob and Monod,⁴ lacZ has been a pillar of many techniques in molecular biology. The lacZ-encoded β-galactosidase enzyme is used universally as a reporter across biological systems for measuring increases in transcription or translation, yeast two-hybrid screens, or monitoring cellular senescence. In Drosophila, lacZ is frequently used to measure manipulations of gene expression. The turnover of β-galactosidase in flies has been reported to be between 5–7 h,⁵ making it ideal to be able to measure temporal changes in expression during aging. In comparison to traditional techniques like western blotting, a β-galactosidase assay is completed within an hour instead of days. Although genomic approaches allow for measurements of gene expression, the sophisticated equipment and elevated cost to execute these experiments often hampers the accessibility to perform such experiments in third world countries or laboratories with limited funding. Here, we present a fast and cheap method (~$0.08CAD/reaction in reagents; $2.07CAD including cost of a microplate) that can be performed with standard laboratory equipment. This protocol describes the steps for measuring Drosophila larvae, pupae, and whole or dissected adults. This assay uses chlorophenol red-β-D-galactopyranoside (CPRG) as substrate, which is reported to be more sensitive than o-nitrophenyl-β-D-galactopyranoside (ONPG), and for fly extracts has the advantage that the product absorbs at a wavelength that does not interfere significantly with eye pigments.⁶ This technique can be used to measure whole animal, dissected tissues or cells. Extracts can be stored at any temperature and enzyme activity perdures for up to three days after extraction (Figure 1).

Preparation of extraction buffer and substrate

© Timing: 10 min
1. Prepare the Extraction buffer. See recipe and storage conditions in the materials and equipment.
   a. Prepare 0.1 mL for every fly being measured, plus the volume needed to prepare the CPRG.
2. Prepare the stock solution of CPRG from powder at 20 mM using Extraction buffer as solvent.
   Note: For long term storage, aliquot and store at \(-20^\circ\)C. Aliquoting by 0.5 mL is the precise amount needed to prepare 10 mL of 1 mM CPRG, which is just enough to fill a standard 96-well microplate.
3. Dilute the CPRG to a working concentration of 1 mM using Extraction buffer as solvent.
   a. Prepare 0.1 mL for every fly being measured.
## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Chemicals, peptides, and recombinant proteins** | | |
| Chlorophenol red-B-D-galactopyranoside (CPRG) | Sigma Aldrich | Cat#59767 |
| Complete, Mini, EDTA-free protease inhibitor cocktail | Roche | Cat#11836170001 |
| Na$_2$HPO$_4$ | BioShop | Cat#SPD307 |
| NaH$_2$PO$_4$ | BioShop | Cat#SPM306 |
| MgCl$_2$ | BioShop | Cat#MAG510 |
| Bovine serum albumin (BSA) | BioShop | Cat# ALB001 |
| **Critical commercial assays** | | |
| Bio-Rad protein assay dye reagent concentrate | Bio-Rad | Cat#5000006 |
| **Software and algorithms** | | |
| SoftMax Pro | Molecular Devices | https://www.moleculardevices.com/products/microplate-readers/acquisition-and-analysis-software/softmax-pro-software |
| **Other** | | |
| SpectraMax 190 | Molecular Devices | Cat#190 |
| Micropipettes | Sigma Aldrich | Cat#Z359947 |
| Corning® 96-well EIA/RIA Clear Flat Bottom Polystyrene Not Treated Microplate | Corning | Cat#9017 |
| Thermo Scientific Medifuge Small Benchtop Centrifuge | Fisher Scientific | Cat#13-101-501 |
| PIPETMAN Classic Starter Kit P20, P200, P1000 | Gilson | Cat#F167300 |

## MATERIALS AND EQUIPMENT

| Extraction buffer | | |
|-------------------|-------|-------|
| Reagent           | Final concentration | Amount |
| Na$_2$HPO$_4$/NaH$_2$PO$_4$ (100 mM pH 7.2)$^1$ | 50 mM | 25 mL |
| MgCl$_2$ (10 mM)$^2$ | 1 mM | 5 mL |
| Complete™ protease inhibitors cocktail tablet (Roche)$^3$ | 1 tablet/50 mL | 1 tablet |
| ddH$_2$O | N/A | 20 mL |
| Total | N/A | 50 mL |

Extraction buffer can be stored at 4°C up to 1 week. 1: Na$_2$HPO$_4$ and NaH$_2$PO$_4$ are stored as 1 M at 23°C–25°C up to 2 years. The 100 mM solution can be stored at 23°C–25°C up to 1 month. 2: MgCl$_2$ is stored at 1 M at 23°C–25°C up to 2 years. The 10 mM solution is stored at 23°C–25°C up to 1 month. 3: Tablets are stored at 4°C up to expiration date.

⚠️ CRITICAL: MgCl$_2$ cannot be omitted. In our experience, most accidental omissions did not impact the assay but in few occurrences the enzyme activity was reduced or abolished.

**Note:** Protease inhibitor is not strictly required but prolongs the enzyme activity over time if stored at −20°C. The effect of storage temperature and protease inhibitor on the longevity of enzyme activity and protein content was assessed (Figure 1). It can be seen that only after 24 h at −20°C does the enzyme activity start to decline (Figure 1A). The addition of the protease inhibitor has a tendency to slow this decline slightly, at least until 48 h. In terms of protein content, no matter the storage temperature the protein content starts to decline after 24 h (Figure 1B).
Alternatives: The SoftMax Pro software and SpectraMax 190 microplate reader can be substituted with any microplate reader and associated software. The Corning 96-well microplates used here can also be substituted with any clear, untreated microplate. Microsoft Excel is used here for data processing, but any spreadsheet software could be used instead such as Apple Numbers or Linux LibreOffice. Any benchtop microcentrifuge can be used instead of the one from Thermo Scientific. Other brands of micropipettes may be used instead, but using Gilson is strongly recommended for their uncontested accuracy and durability.

Optional: A Microsoft Excel template is provided which if desired can be used to calculate the preparation of all solutions used, provides a blank table in which to copy raw data, graphs the data, and calculates specific activity automatically. Two versions of the template are provided in supplemental information, a version which is empty (Data S1) and ready to be used, and another which has been filled with sample data (Data S2) to demonstrate how the template works.

STEP-BY-STEP METHOD DETAILS

Extract preparation

© Timing: 1–2 h

Collect and grind fly in extraction buffer.

1. Aliquot 100 μL of Extraction buffer into 1.5 mL microcentrifuge tubes. Prepare as many tubes as the number of organisms to be processed.

   Note: Generally, five individuals of a given genotype or treatment are measured per experimental replicate. The variability resulting from pipetting error is negligible (<1.5%), however performing reactions in duplicate or triplicate may be necessary for inexperienced experimentalists, or when using low grade or homemade micropipettes. At least four experimental replicates are measured and presented data represent the average of all individuals across all four replicates (Figure 2).

2. Collect samples to be measured and deposit one organism into each microcentrifuge tube of Extraction buffer.

   Note: Whole organisms may be used, or specific body parts may be dissected. Here, whole L3 larvae and early pupae were used and adult thorax dissections were performed as follows.

   a. Use anesthesia to knock out the flies.
   b. Use forceps to grasp the legs and position the fly.
   c. Use a scalpel to sever the head and abdomen.
   d. Using the forceps to deposit one dissected thorax into each microcentrifuge tube of Extraction buffer.

3. Grind the tissue.

   a. Using a micropestle grind the thorax by hand in the Extraction buffer until it is clearly broken apart.

   Note: Try and be as consistent as possible with grinding the tissue samples. For example, apply the same number of turns (or strokes) of the micropestle. It does not take great effort or time to grind fly samples, and only requires visual inspection to confirm that the sample has been broken apart. Using mechanical homogenizers are not necessary, unless you are working with another organism that has more robust tissue.
4. Centrifuge the extracts at 10,000 g for 1 min.

△ CRITICAL: Positive and negative controls. It is critical to include a sample that is known to have no activity as a negative control. The residual activity that may be observed in some
situations should be subtracted from the activity of the experimental extracts. The detection of significant activity in the negative control would happen if the pH of the Extraction buffer is incorrect (See troubleshooting, problem 2). When the bipartite UAS/GAL4 system is used to express lacZ, negative control genotypes are generated in which one component is missing (UAS alone). It is also important to include a positive control sample. In Drosophila there are a multitude of genotypes that are easy to obtain or generate. We routinely use lacZ enhancer trap strains that do not require any crosses, such as arm-lacZ (BDSC: 7370). In other systems one could acquire purified β-galactosidase preparations, E. coli extracts or saliva.

Note: Cold temperature for extraction is not critical. Both enzyme activity and protein content perdure for hours at 23°C–25°C. Freezing diminishes enzyme activity and protein content declines no matter the temperature after 24 h (Figure 1).

Alternatives: Since lacZ expression is restricted to the adult muscle in the samples used, thorax dissections were performed in order to enrich extracts and reduce variability caused by the inclusion of non-expressing tissues. Dissection procedures may be altered or omitted depending on specific needs.

Enzyme measurement

- Timing: 1 h

Fill the microplate and measure using the spectrophotometer with microplate reader.

5. Set the microplate reader.
   a. Kinetic measurements (every 30 s, for 30 min) at wavelength 562 nm.

6. For each extract, pipette the volume of extract to be tested (2–10 μL) into a well of a microplate.

   Note: This range of volumes is appropriate for 100 μL Drosophila extracts, but the exact volume may have to be adjusted for samples with very high activity. The extraction volume and the volume to measure will be determined in a pilot experiment when extracts are made from other sources.

7. Distribute 100 μL of 1 mM CPRG into each well.

   Note: Since the volume of CPRG is at least ten times the volume of extract, mixing is not strictly required. When the option is available the microplate reader can be configured to shake the microplate to ensure reactions are well combined.

8. Place the microplate in the reader and hit Start.

9. After the measurements have all been taken, the data can either be exported as a plain text (.txt) format and copied into a desired spreadsheet software and proceed to steps 10 and 11.

   Optional: Alternatively, export the data as a plain text (.txt) format and copy the data into the optional LacZ template empty.xls file (Data S1) and proceed to Optional steps for automated data processing.

10. Plot the readings for each extract (Absorbance vs Time).

11. Calculate SAV, the enzyme specific activity per volume of extract (ΔA/time/volume). The calculations are done to get the activity in ΔmA/min/mL (change in milli-absorbance per minute per mL of extract). In all below equations units of each variable are indicated in square brackets.
a. Evaluate the ‘Absorbance vs Time’ plots. Positive controls should show activity and negative controls should show little or no activity. Figure 3 shows an example of an extract with no activity.

b. For all measurements, select points from the ‘Absorbance vs Time plot’ where the curve is linear (close to the start of the reaction). Figure 4 shows an example of selecting points from a plot where curve plateaus. Calculate the enzyme activity (EA) as the change in absorbance per minute ($\Delta A$/min) using the slope between the two points:

$$EA = \frac{Absorbance\ at\ time\ 2 - Absorbance\ at\ time\ 1}{t_2 - t_1} = \frac{A_2 - A_1}{t_2 - t_1}\ \text{mU}$$

We convert the unit of EA from $\Delta A$/min to the change in milli-absorbance per minute ($\Delta mA$/min) by multiplying the EA by 1000. This is optional but is done to avoid working with values with a large number of decimal places.

$$EA\ [\Delta mA / min] = EA\ [\Delta A / min] \times 1000$$

c. Calculate the specific enzyme activity per volume (SAV) in $\Delta mA$/min/mL:

$$SAV = \frac{EA [\Delta mA / min]}{volume\ of\ extract\ measured\ [mL]}$$

d. Average the SAV from the negative controls. This represents the background activity BSAV.

e. Subtract the background activity from each experimental measurement to obtain the corrected enzyme activity CSAV.

Note: To ensure that the rate of the reaction is not influenced by the concentration of substrate and instead is only influenced by the concentration of enzyme in the extract, 1 mM
CPRG (step 7) is used. The reaction is initiated as soon as CPRG is added. For a full microplate one would perform pilot measurements to determine the volume of extract appropriate to set reaction rates tailored to the speed of the experimentalist.

When measuring genotypes where no activity is expected, as in negative controls, it is important to be able to distinguish between a reaction that yields low levels of activity, versus no activity. It is important to take a sufficient number of readings (step 5a) to be sure of the shape of the curve. Especially, at such low levels there can be “noise” or slight fluctuations in the readings that may appear like activity when only looking at very few points. Figure 3 demonstrates an example of a reaction with activity versus one without.

It is also very important to ensure the selection of the points used in the activity calculations is done accurately (step 11b). With high concentrations of enzyme, it is possible for the reaction to plateau. It would be inappropriate to take the linear regression through the entire curve, as it would underestimate the true level of activity. Figure 4 demonstrates how to select points in a situation where the curve plateaus. It is most accurate to take points as close to the start of the reaction as possible.

Optional: The length of measurement time, and the number of readings taken can be revised for specific needs (step 5a). The volume of extract measured (step 6) can be revised depending on the concentration of samples being measured. The following steps describe how to use the optional Lac template empty.xls file (Data S1) (step 9):

i. The empty template file and a filled template file with example data are provided in the supplemental information. In the provided template, all cells that require information to be filled are highlighted in orange. Enter sample names how they will be loaded in the plate in the ‘Raw data table.’ The number of samples will be calculated automatically and used to automatically calculate the preparations of the Extraction buffer, Substrate, and Bradford dye. All cells with values to be pipetted are highlighted in green.

ii. Enter the mass of BSA weighed for stock preparation. The volume of solvent to add will be automatically calculated.

Figure 4. Selecting data points to calculate enzyme activity
Typical example of activity measurement plotted as absorbance over time from an extract with high activity. Since the plot of the activity is not linear, points are selected near the start of the reaction where the slope is linear (highlighted in red).
iii. Enter names for the BSA standard curve dilutions and the volume prepared of each. The volumes to pipette will be automatically calculated.

iv. Once the CPRG assay has run, export the data in a plain text format (.txt). Copy the absorbance readings into the Raw data table in the template. The data will be plotted automatically in graphs at the bottom of the table.

v. At the bottom of the Raw data table there are three additional rows ‘Start,’ ‘End,’ and ‘$R^2$.’ These rows are used to determine the appropriate data points which will be used to calculate the slope of the enzyme activity curves. Enter time points in the ‘Start’ and ‘End’ rows. Looking at the graphs should give an idea of where the curves are linear and where they start to plateau. The $R^2$ value (square of Pearson product-moment correlation coefficient) is calculated to test the linearity between the selected ‘Start’ and ‘End’ points. Perfect linearity will give a value of 1. The $R^2$ value will be calculated automatically. Non-adjacent points must be selected to assess linearity with an $R^2$ value. The cut-off value of 0.999 is used. Conditional formatting has been applied to highlight $R^2$ values that are <0.999, which would indicate the slope is changing (plateauing). If the returned $R^2$ value is highlighted, select new time points.

vi. Below the Raw data table are the CPRG and Bradford data analysis tables. The activity will automatically be calculated under the ‘$\Delta$mA/min/mL’ heading using the time points selected in the ‘Start’ and ‘End’ rows.

Protein quantification

© Timing: 1 h

Perform Bradford assay for quantification of protein content in extracts.

12. Prepare a standard curve using BSA.
   a. Prepare a stock solution of BSA at 2 mg/mL.
   b. Perform 2-fold serial dilutions to prepare 1, 0.5, 0.25 and 0.125 mg/mL BSA solutions that will be used to generate the standard curve. Use Extraction buffer as the 0 mg/mL BSA.

13. Prepare the Bradford dye from concentrate (Bio-Rad Protein Assay Dye Reagent Concentrate).
   a. Dilute concentrate 5-fold. Prepare 0.2 mL for each concentration in the standard curve, plus each extract to be quantified.

14. For each extract, pipette the volume of extract to be measured (2–10 $\mu$L) into a well of a microplate.

15. For each concentration of the standard curve, pipette 10 $\mu$L into a well of a microplate.

16. Distribute 200 $\mu$L of diluted Bradford dye into each well.

17. Incubate at 23°C–25°C for at least 5 min before measuring.

△ CRITICAL: Incubation time must not exceed 1 h, as the dye-protein complex will precipitate.

18. While incubating, set the microplate reader.
   a. Single measurement at wavelength 595 nm.
   b. After incubation, place the microplate in the reader and hit Start.

19. If using a preferred spreadsheet software, export the data in a plain text format (.txt) and use steps 20 and 21 to calculate the specific activity.

   **Optional:** If using the optional LacZ template empty.xls file (Data S1), export the data in a plain text format (.txt) and proceed to Optional steps below for automated data processing.

20. Determine the protein concentrations of the extracts. The below calculations compute the concentration in mg/mL.
a. Correct the absorbance readings of the Bradford assay. Subtract the absorbance of the 0 mg/mL BSA sample from all measurements. The new values represent the corrected absorbance readings. The 0 mg/mL BSA sample should have an absorbance of 0.

b. Create a plot of the standard curve with the corrected absorbance readings of the BSA samples vs concentration (0, 0.125, 0.25, 0.5, 1 mg/mL).

c. Fit the corrected absorbances from each of the extracts to the standard curve to determine the concentration (FC).

d. Obtain the protein concentration (PC) by multiplying with the ratio of BSA sample volume to extract volume:

\[ PC\text{[mg/ml]} = FC\text{[mg/ml]} \times \frac{\text{volume of BSA measured [ml]}}{\text{volume of extract measured [ml]}} \]

21. Calculate the specific activity (ΔmA/min/mg).

\[ \text{Specific activity [ΔmA/min/mg]} = \frac{\text{CSAV[ΔmA/min/mL]}}{PC\text{[mg/ml]}} \]

Optional: The volume of extract measured (step 14) can be revised depending on the concentration of samples being measured. All absorbances should plug between experimental points of the standard curve. The following steps describe how to use the optional Lac template empty.xls file (Data S1) (step 19):

vii. In the Bradford data analysis table, the loading order can be entered by filling the sample names in the ‘Sample’ column in correspondence with the ‘Well’ column. The names of the samples in the BSA standard curve should match the names given at the top in the BSA dilutions preparation table.

viii. Once the Bradford has run, export the data in a plain text format (.txt). Copy the absorbance readings into the Bradford data analysis table under the heading ‘Unblanked.’

ix. At the very bottom of the Bradford data analysis table is the Standard curve. The template will automatically extract the absorbance readings for the BSA dilutions from the ‘Unblanked’ column based on the assigned names of the BSA dilutions in the ‘Sample’ column. The absorbances for the BSA dilutions will be entered into the Standard Curve table automatically.

x. The ‘Corrected’ column takes into consideration the blank (BSA 0 mg/mL), subtracting this absorbance from the absorbance of every sample.

xi. A graph of the Standard Curve is produced automatically using the corrected absorbances vs protein concentration.

xii. In the ‘Plug to curve’ column of the Bradford analysis table, the corrected absorbances for each sample are plugged into the Standard Curve. The formula will test between which pair of points in the standard does the point plug. Then using the trend function, the concentration is obtained by plugging the absorbance to the equation of the line between said points.

xiii. The final concentration is given in the ‘mg/mL’ column, where the last step is to take the value that was given by fitting to the curve and adjust based on the ratio of the volume of BSA standard loaded to the volume of sample that is loaded.

xiv. Specific activity will be automatically calculated (‘ΔmA/min/mg’ column) by looking up the sample by name and dividing the activity in ΔmA/min/mL by the concentration in mg/mL.
EXPECTED OUTCOMES

We present here a protocol for preparing extracts and quantifying β-galactosidase specific activity in a highly accessible, cost-effective manner. These assays have been performed routinely in our lab for over 20 years, by many different experimentalists. The data presented were collected by two different experimentalists and the results are reproducible even if they are measured decades apart. As previously reported, DJ694 expression is higher in females than males and Mef2 expression is higher in the adults than in the third instar larvae and early pupae (Figure 2).

Here, we measure activity in whole animals or dissected thoraces, but this assay can certainly be used with any model organism, isolated tissue or cells. This assay has been successfully performed on tissues samples with a low number of cells such as *Drosophila* antennae. When performing this assay on tissue with fewer cells, in order to concentrate the sample enough to see a signal, one can reduce the volume of Extraction buffer used to grind. However, this might make grinding more challenging. Alternatively, using more than one animal per extract works well. We have taken this approach when measuring first instar *Drosophila* larvae but it has the inconvenience that it increases variability due to the use of multiple individuals. Of course, when measuring any sample for the first time and the concentration of β-galactosidase is unknown, testing different volumes and/or concentrations of extract is required to obtain a measurable signal.

LIMITATIONS

There are two main contributors to variability in these measurements. The first comes from inconsistency in the grinding of the tissue samples, resulting in differences in the yield of extraction. It is critical to be as consistent as possible when grinding the tissue. A second source of variability depends on the genotypes being measured and how expression changes across time. If lacZ expression level changes greatly during certain time periods, in order to reduce variability between samples it would be pertinent to keep time windows for staged organisms as small as possible.

TROUBLESHOOTING

Problem 1
No activity is detected where there should be.

Potential solution
Having no activity is unlikely the result of a pH issue from an error with the preparation of the phosphate buffer, as the specific pH of 7.2 is used in order to differentiate activity from the endogenous lacZ versus activity from the exogenous lacZ expression. The original recipe for the Extraction buffer is derived from Simon and Lis. The potassium phosphate buffer was substituted with a sodium phosphate buffer to be amenable with procedures that involve sodium dodecyl sulfate (SDS), as potassium reacts with SDS to form flocculating potassium salt of SDS. A phosphate buffer is used as it has a pKa of 7.2. If the pH was more acidic such that the conditions were permissive to the endogenous activity, higher activity would be expected. Additionally, an incorrect pH would result in the detection of activity in the negative control.

The more likely cause of no activity would be if MgCl₂ had been omitted from the Extraction buffer. In our experience, most accidental omissions did not impact the assay but in few occurrences the enzyme activity was reduced or abolished. Prepare fresh Extraction buffer with MgCl₂ (preparation of extraction buffer and substrate, step 1).

Problem 2
The negative control is positive.

Potential solution
It is important to ensure the smooth pipetting of the CPRG into the microplate to avoid splashing which may lead to contamination of the negative control (enzyme measurement, step 7). Significant
activity in the negative control will occur if the pH of the Extraction buffer is incorrect and the sample is known to contain endogenous β-galactosidase (See troubleshooting, problem 1).

**Problem 3**

Concentration of β-galactosidase is too low to be detected.

**Potential solution**

Since the activity of extracts can perdure for several days even at 23°C–25°C (Figure 1), it is unlikely that the lack of activity would be attributed to sample degradation. The protocol does rely on availability of enough amount of tissue. For samples where the volume of tissue is relatively small (i.e., certain small, dissected body parts, Drosophila embryos, or L1 larvae) more than one organism can be measured per sample (extract preparation, step 2).

**Problem 4**

The Absorbance vs Time curves plateau immediately.

**Potential solution**

The concentration of β-galactosidase is too high. Decrease the volume of extract measured or dilute the extract with Extraction buffer (enzyme measurement, step 6).

**Problem 5**

A measurement that had been performed previously does not repeat as expected.

**Potential solution**

When using a frozen stock of CPRG, upon thawing some precipitation can occur (color gradient is clearly visible). Inexperienced experimentalists may neglect to vortex the thawed solution, resulting in a concentration of CPRG that is well below 1 mM (preparation of extraction buffer and substrate, step 3). This would result in an enzyme activity that is much lower than expected since the binding of the substrate will influence the rate of the reaction.

Storage conditions of extracts may impact enzyme activity or protein content. At –20°C after 24 h, enzyme activity begins to decline (Figure 1A). Protein content is observed to be lower after 24 h no matter the storage temperature (Figure 1B). Lower protein content would result in elevated specific activity. Being consistent with storage of extracts is necessary for reproducible measurements.

**RESOURCE AVAILABILITY**

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Laurent Seroude (seroudel@queensu.ca).

**Materials availability**

This study did not generate any unique reagents.

**Data and code availability**

Figures 1 and 2 data have been deposited at Mendeley data and are publicly available Data 1 (Mendeley Data: https://doi.org/10.17632/vx8x5fx95n.1) and Data 2 (Mendeley Data: https://doi.org/10.17632/k2m2m3jgmn.1) respectively.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101843.

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AUTHOR CONTRIBUTIONS
Conceptualization, T.B. and L.S.; Methodology, T.B. and L.S.; Validation, T.B. and L.S.; Writing - Original draft, T.B.; Writing - Review & Editing, T.B. and L.S.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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