**Standard Operating Procedure:   
Biochemical Analyses of Catalase (with parallel BCA protein determination)**

CM, CYW  
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Date of last revision: February 14th, 2025

**Kit:** Invitrogen™ Catalase (CAT) Colorimetric Activity Kit, 2 x 96 Tests, Catalog # EIACATC

\*Note: UPON RECEIPT, KEEP KIT IN FRIDGE (4°C) UNTIL USE. DO NOT FREEZE.

**Additional materials needed but not supplied:**

* Plate reader (CAT: 560 nm, Protein: 562 nm)
* Precision pipettes (8 channel, 10-200 uL; single channel, 10-200uL; single channel, 5mL)
* 1.5 mL tubes for creating serial dilution of standards and diluting samples
* 10, 100 mL tubes for preparing reagents (1x Assay Buffer, 1X Horseradish peroxidase solution)
* Optional: Incubator (20°C)
* Supernatant for all samples (frozen): 125-150uL
* DI water

1. Remove kit from fridge and allow to reach room temperature.   
   Place samples from freezer into fridge to thaw.
2. Prepare reagents

Note: Reagent volumes are per plate as we carried at one plate per day. 2 plates total needed for all samples. We carried out plates over 2 consecutive days

1x Assay Buffer (diluent)

1. Prepare and label 100 mL tube (Label: 1x Assay Buffer).
2. Dilute 14 mL of Assay Buffer (5x) with 56 mL of distilled water.
3. Vortex to ensure good mixing.
4. Store 5x Assay Buffer concentrate and 1x Assay Buffer in fridge for up to 3 months.

1X HRP solution (25 uL per well)

1. Prepare and label 10 mL tube (Label: 1X HRP).
2. Vortex Horseradish Peroxidase Concentrate (HRP) (50x) prior to pipetting. Pipette from bottom of the tube.
3. Add 50 uL of HRP (50x) to 2.45 mL of 1x Assay Buffer (=2.5 mL).
4. Vortex to ensure good mixing.

Note: 1X HRP stable for one day only. Allow to warm to room temperature prior to application to plate.

1. Blank plate (560 nm) prior to preparation of samples and standards.  
   Note: not sure this is necessary but did just in case.
2. Prepare samples:
3. Remove samples from fridge and leave on benchtop to warm to room temperature.
4. Dilute samples in 1X Assay Buffer as per pre-determined dilution factor

Note: We trialled dilution factors of 0, 1:4 (Plate 1), and 0, 1:24 (Plate 2). We found that 0 and 1:4 absorbance values fell in high range regardless of DF; We had better success with 1:24; propose NOAA try 1:24 (for direct comparison) and 1:50 or 1:100.

* Sample volume is 25 yl per well. two samples (in duplicate) may need more than 50 ul with pipetting error, below for 3 ul total 70 ul for 1:24 dilution.
* Note: requires 72 ul of 1x assay buffer, make sure there’s enough!
  + 120 samples \* 72 ul = 8,630 ul (8.64 mL) 1X Assay buffer
* Sample and standards (plate layout notes)
  + - 60 total samples in duplicate (120 wells)
    - 7 standards run in duplicate (14 wells)
    - Plate 1 (# wells):
      * 14 standard wells (7 standards)
      * 82 sample wells (41 samples)
    - Plate 2 (# wells):
      * 14 standard wells (7 standards)
      * 38 sample wells (19 samples)

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AI-generated content may be incorrect.

1. Vortex samples to ensure mixing.
2. Use samples within **2 hours of preparation**.
3. Prepare standards (refer to production information sheet):

Note: We ran 1 set of standards per plate and made new standards for each of the two plates (as ran over more than one day).

1. Prepare and label 7 x 1.5 mL tubes: 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 U/mL SOD (Stds 1-7)
2. Add 190 uL 1x Assay Buffer to 5 U/mL tube (Std 1) ONLY and 100 uL 1x Assay Buffer to remaining 6 tubes (Stds 2-7).
3. Add 10 uL Catalase Standard to 5 U/mL tube (Std 1) ONLY. Mix thoroughly.
4. Carry out serial dilution as shown on P.2 of the production information sheet. Add 100 uL of Std 1 to Std 2. Mix thoroughly. Repeat for Stds 3-6, but NOT Std 7 (0 U/mL). Mix thoroughly between steps.
5. Use standards within **2 hours of preparation.**
6. Prepare plate:

Note: You will require standards, diluted samples, Hydrogen Peroxide Reagent, Substrate, 1X HRP.

All reagents, standards, and samples should be at room temperature prior to use. Mix well prior to use.

->First incubation

1. Add 25 uL of standards and samples in duplicate to plate as per plate layout below.
2. Add 25 uL of Hydrogen Peroxide Reagent into each well.
3. Incubate for 30 minutes at room temperature.

->Second incubation

1. Add 25 uL of Substrate into each well.
2. Add 25 uL of 1x HRP into each well.
3. Incubate plate for 15 minutes at room temperature.
4. Read plate absorbance (560 nm).

Catalase Plate Layout:

7 standards, 3 treatments (Ctrl, OA, HW (plus baseline (BL)); 3 timepoints (T1=20°C, T2=24°C, T3=24 hr recovery) per treatment (e.g. T1OA, T2OA T3OA); 6 samples per treatment per timepoint (1-6), 2 replicates per samples (1,1).

PLATE 1

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Std 1 | 1 | T1Ctrl1 | 1 | T2Ctrl1 | 1 | T3Ctrl1 | 1 | T1OA 1 | 1 | T2OA 1 | 1 |
| B | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| C | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| D | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| E | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| F | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| G | 7 | 7 |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

PLATE 2

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Std 1 | 1 | T3OA 1 | 1 | T1HW1 | 1 | T2HW1 | 1 | T3HW1 | 1 | BL 1 | 1 |
| B | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| C | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| D | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| E | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| F | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| G | 7 | 7 |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

**BCA Protein Determination for CAT samples**

**Kit:** BCA Protein Pierce™ BCA Protein Assay Kit, 500mL, 10 x 1 mL glass ampules, 250 tubes or 2,500 microplate assays, Catalog number [23227](https://www.thermofisher.com/order/catalog/product/23227)

\*UPON RECEIPT, STORE KIT AT ROOM TEMPERATURE.

**Additional materials needed but not supplied:**

* 1x assay buffer (from CAT kit) for diluent
* 96 micro-well plates
* Plate reader (562 nm)
* Plate shaker
* Precision pipettes (8 channel, 10-200 uL; single channel, 10-200uL, single channel, 5mL)
* 1.5 mL tubes for creating serial dilution of standards and diluting samples
* 50 mL tubes for BCA working reagent and CAT 1x Assay Buffer (standard diluent)
* Incubator (set to 37°C).

USE Microplate procedure (sample to WR ratio = 1:8)

1. Turn on incubator (set to 37°C).
2. Move samples from freezer into fridge to thaw.
3. Prepare Standards
4. Prepare standards according to Table 1 of User Guide using 9 x 1.5 mL tubes. Label A-I. Keep at room temperature.

Note: For catalase protein determination, use CAT kit 1x Assay Buffer as diluent.

1. Prepare samples:
2. Remove samples from fridge and leave on benchtop to warm to room temperature.
3. If necessary, dilute samples in 1X Assay Buffer as per per-determined dilution factor

Note: We did not dilute our samples but propose using 1:1 or 1:2 dilutions.

1. Vortex samples to ensure mixing.
2. Use samples within **2 hours of dilution**.
3. Prepare BCA working reagent (WR)

Note: Reagent volumes are for 2 plates as we carried at both plates (covering all samples) in one day.

1. Determine volume of BCA working reagent (WR): (#standards + #samples) x (#replicates) x (200uL) (See User Guide). Therefore, (9+60) x (2) x (200) x 2 plates = ~55 mL (Note: we made 65 mL).
2. Make up WR at a ratio of 50:1 (Reagent A : Reagent B). For 65 mL, mix 65 mL of Reagent A with 1.3 mL Reagent B

Note: WR is stable for several days in a closed container at room temperature.

1. Prepare Plate
2. Pipette 25 µL of each standard or sample replicate into a microplate well (working range = 20–2000 µg/mL) according to plate layout below.
3. Add 200 µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
4. Cover the plate and incubate at 37°C for 30 minutes.
5. Equilibrate the plate to room temperature prior to reading (5-10 minutes).
6. Measure plate absorbance (562 nm).

Protein Plate Layout:

9 standards, 3 treatments (Ctrl, OA, HW (plus baseline (BL)); 3 timepoints (T1=20°C, T2=24°C, T3=24 hr recovery) per treatment (e.g. T1OA, T2OA T3OA); 6 samples per treatment per timepoint (1-6), 2 replicates per samples (1,1).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Std A | A | T1Ctrl1 | 1 | T2Ctrl1 | 1 | T3Ctrl1 | 1 | T1OA 1 | 1 | T2OA 1 | 1 |
| B | B | B | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| C | C | C | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| D | D | D | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| E | E | E | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| F | F | F | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| G | G | G | H | H | I | I |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Std A | A | T3OA 1 | 1 | T1HW1 | 1 | T2HW1 | 1 | T3HW1 | 1 | BL 1 | 1 |
| B | B | B | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| C | C | C | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| D | D | D | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| E | E | E | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| F | F | F | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| G | G | G | H | H | I | I |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |