Optimized protocol for brain and head kidney catalase activity in zebrafish

Caio Maximino,Bruna Patrícia Dutra Costa,Gabriel Rocha Felício

Abstract

Zebrafish, and other small teleosts, are used as experimental models to evaluate human pathologies, including those linked to oxidative stress. The protocol presents an optimized technique to evaluate the activity of catalase, an important antioxidant enzyme, in zebrafish tissues, focusing on the brain and head kidney. The protocol is based on the classical Aebi (1984) method.

Citation: Caio Maximino,Bruna Patrícia Dutra Costa,Gabriel Rocha Felício Optimized protocol for brain and head kidney catalase activity in zebrafish. **protocols.io**
dx.doi.org/10.17504/protocols.io.rumd6u6
Published: 01 Aug 2018

Guidelines

This protocol is intended to analyze catalase activity in zebrafish brain and head kidney tissue samples. It can be adapted for other fish species or other tissues without much hassle; however, tissue amounts need to be adjusted.

Before start

Every biochemical protocol needs to be validated in the laboratory when first introduced. The present protocol describes validation steps that were taken in LaNeC.

Materials

- Hydrogen peroxide by Contributed by users
- Disposable polystyrène cuvettes (1ml) 786-009 by G-Biosciences
- 12x75mm test tubes 0555512 by Fisher Scientific
- Double-bean UV-visible spectrophotometer, with temperature control in the cuvette compartment by Contributed by users
- Phosphate buffer (0.1 M, pH 7.3) [80 ml 0.1 M Na2HPO4 (13.8 g Na2HPO4·H2O/liter dH2O) 20 ml 0.1 M NaH2PO4 (26.8 g NaH2PO4·7H2O/liter dH2O)] View by Contributed by users

Protocol

Assay validation: Linearity

Step 1.

Prepare reagents for the linearity curve:

1. Prepare phosphate buffer (0.1 M, pH 7.3)

2. Dilute 0.34 ml 30% hydrogen peroxide (H_2O_2) with phosphate buffer to 100 ml, reaching a final concentration of 30 mM H_2O_2.

3. Make seven serial dilutions of 30 mM H_2O_2 (down to 0.234375 mM) in phosphate buffer

REAGENTS

- Phosphate buffer (0.1 M, pH 7.3) [80 ml 0.1 M Na2HPO4 (13.8 g Na2HPO4·H2O/liter dH2O) 20 ml 0.1 M NaH2PO4 (26.8 g NaH2PO4·7H2O/liter dH2O)] View by Contributed by users
- Hydrogen peroxide 30% 822287.1000 by Merck Millipore
- 12x75 mm high clarity polypropylene test tubes 352063 by Corning

Assay validation: Linearity

Step 2.

H_2O_2 quantification

1. Allow reagents to equilibrate, and pour in a Quartz cuvette.

2. Read on a spectrophotometer at 240 nm.

NOTES

ATTENTION

All analyses should be made with at least three technical replicates

Thoroughly rinse the cuvettes with distilled water to remove contaminants before each replicate

Assay validation: Linearity

Step 3.

Data should be entered in two columns, 'x' and 'y', representing concentration and response, respectively. Plot data and perform a linear regression:

SOFTWARE PACKAGE (Xubuntu -)

chemCal for R, 0.2.1

Johannes Ranke (https://orcid.org/0000-0003-4371-6538)
https://cran.r-project.org/web/packages/chemCal/index.html

cmd COMMAND

```
if(!require(chemCal)){
   install.packages('chemCal')
   library(chemCal)
}

m <- lm(y ~ x, data = your_data)

summary(m)

calplot(m)
```

Determine linearity and curve parameters using chemCal for R

Assay validation: Linearity

Step 4.

Correlation coefficients higher than 0.98 suggest linearity. Error residuals should also be checked, looking for deviations in linearity, presence of atypical samples, heteroscedasticity, and dependence between errors; a well-adjusted curve should present erros with a uniform distribution, average zero and constant variance, and absence of atypical samples. An F-test comparing fits of residuals with a linear vs. quadratic model can also be used.

Assay validation: Sensitivity

Step 5.

The first step in determining the sensitivity of the assay is to determine the limit of detection (LOD). This can be done using parameters from the linear model, with the function 'lod' from the R package chemCal. Use alpha = 0.01 and beta = 0.5.

SOFTWARE PACKAGE (Xubuntu -)

chemCal for R, 0.2.1

Johannes Ranke (https://orcid.org/0000-0003-4371-6538)
https://cran.r-project.org/web/packages/chemCal/index.html

cmd COMMAND

```
lod(m, alpha = 0.01, beta = 0.5)
```

Calculate limit of detection using chemCal for R

Assay validation: Sensitivity

Step 6.

The next step in determining sensitivity of the assay is to determine the limit of quantification (LOQ). This can also be done using parameters from the linear model, with the 'loq' function from the package chemCal. Substitute 'your_n' by the number of technical replicates in the your assay.

SOFTWARE PACKAGE (Xubuntu -)

chemCal for R, 0.2.1

Johannes Ranke (https://orcid.org/0000-0003-4371-6538)
https://cran.r-project.org/web/packages/chemCal/index.html

cmd COMMAND

```
loq(m, n = your_n)
```

Determine limit of quantification with the chemCal package

Assay validation: Precision

Step 7.

Precision refers to the dispersion of measured values around an average value, and its numerical

value is determined by the relative standard deviation (RSD):

$RSD = 100 * s / |\bar{x}|$
Where:
s = the sample standard deviation
$\bar{x}$ = sample mean

Assay validation: Precision

Step 8.

The first step in determining the precision is to assess the repeatability of the assay (that is, intra-run precision). Run three technical replicates for the lowest, intermediate, and highest H_2O_2 concentrations. Determine intra-run precision by calculating the RSD for each of these concentrations. RSDs higher than 15% indicate low precision.

Assay validation: Precision

Step 9.

Intermediate precision, or inter-run precision, defines the precision of a measurement made in the same laboratory made by different analysts and/or different days. We follow ANVISA's recommendations by running three technical replicates for the lowest, intermediate, and highest H_2O_2 concentrations in two different days and assessing the RSD for each concentration. RSDs higher than 15% indicate low intermediate precision.

Assay validation: Accuracy

Step 10.

Accuracy can be determined after the establishment of linearity, sensitivity, and precision. Run another calibration curve, with at least three technical replicates, for the entire range of concentrations. Accuracy is calculated as the difference between predicted (xi) and measured (x_v) absorbances

Predicted values can be obtained based on the linearity curve (Step 1) using a weighted linear model, with weights derived from the curve made for the accuracy determination. Using chemCal (where *new_data* refers to the curve made for accuracy determination)

SOFTWARE PACKAGE (Xubuntu -)

chemCal for R, 0.2.1

Johannes Ranke (https://orcid.org/0000-0003-4371-6538)
https://cran.r-project.org/web/packages/chemCal/index.html

cmd COMMAND

```
weights <- with(new_data, {
  yx <- split(y, x)
  ybar <- sapply(yx, mean)
  s <- round(sapply(yx, sd), digits = 2)
  w <- round(1 / (s^2), digits = 3)
})
new_data.means <- aggregate(y ~ x, new_data, mean)

n <- lm(y ~ x, w = weights, data = new_data.means)
```

```
inverse.predict(new_data.means, 30, ws = your_weight) #your_weight determined in previous s
tep for 30 µM
inverse.predict(new_data.means, 15, ws = your_weight) #your_weight determined in previous s
tep for 15 µM
inverse.predict(new_data.means, 7.5, ws = your_weight) #your_weight determined in previous
step for 7.5 µM
inverse.predict(new_data.means, 3.75, ws = your_weight) #your_weight determined in previous
 step for 3.75 µM
inverse.predict(new_data.means, 1.875, ws = your_weight) #your_weight determined in previou
s step for 1.875 µM
inverse.predict(new_data.means, 0.9375, ws = your_weight) #your_weight determined in previo
us step for 0.9375 µM
inverse.predict(new_data.means, 0.46875, ws = your_weight) #your_weight determined in previ
ous step for 0.46875 µM
inverse.predict(new_data.means, 0.234375, ws = your_weight) #your_weight determined in prev
ious step for 0.234375 µM
```

Generate predicted values from weighted linear model (weights based on the results from the linearity assay)

Assay validation: Accuracy

Step 11.

Use the predicted values x_i obtained in the previous step to calculate the accuracy at each level, as:

Accuracy = ([xi - x_v] / x_v) * 100

Biological sample preparation

Step 12.

Sacrifice animal with ice-cold water (< 12 ºC) followed by spinal transection.

Biological sample preparation

Step 13.

Carefully dissect the brain (http://dx.doi.org/10.3791/1717) and head kidney (http://dx.doi.org/10.3791/2839).

LINK:
http://dx.doi.org/10.3791/2839

Biological sample preparation

Step 14.

Homogenize the tissue in 2 ml PB (0.1 M, pH 7.3)

REAGENTS

Phosphate buffer (0.1 M, pH 7.3) [80 ml 0.1 M Na2HPO4 (13.8 g Na2HPO4·H2O/liter dH2O) 20 ml 0.1 M NaH2PO4 (26.8 g NaH2PO4·7H2O/liter dH2O)] View by Contributed by users

NOTES

Tissues can be kept frozen (- 20 ºC) for up to 6 months.

Measure catalase activity

Step 15.

Re-suspend the homogenate by vortexing, if needed

Measure catalase activity

Step 16.

Carefully transfer the homogenate to the cuvette.

Measure catalase activity

Step 17.

Add 1 ml H_2O_2 (30 mM) solution. Immediately record

Warnings

Make sure to read all Safety Data Sheets for the reagents. Hydrogen peroxide causes serious eye damage; is harmful if inhaled and may cause respiratory irritation; and causes skin irritation. Therefore, use personal protective equipment whenever manipulating it. Moreover, hydrogen peroxide must be manipulated under a fume hood at all times.