NaPDI Repository Data Entry SOP: In vitro Enzyme Induction Studies

Version 1

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# Background

* 1. Scope

The purpose of this Standard Operating Procedure (SOP) is to describe how to enter *in vitro* enzyme induction results into the NaPDI repository. Natural Products (NPs) are expected to be evaluated as causative agents of induction (*Precipitants*). The victim drugs (*Objects*) are probe substrates of known enzymes.

Most of the information entered in the repository will come directly from the study report; avoid interpretations of the authors’ conclusions. However, several text fields are provided throughout the admin site to allow the addition of relevant comments that may pertain to the experimental study design and conditions, the study results, and/or the mechanism of induction. This additional information should be reviewed with the principal investigators during the validation process as it will be used to enrich the users experience and understanding of the results.

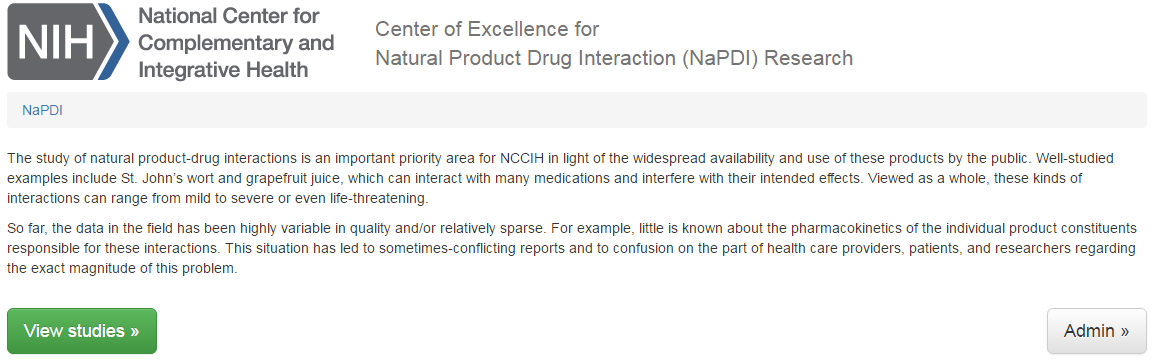
* 1. Definitions

Add user-centered definitions (alphabetically)

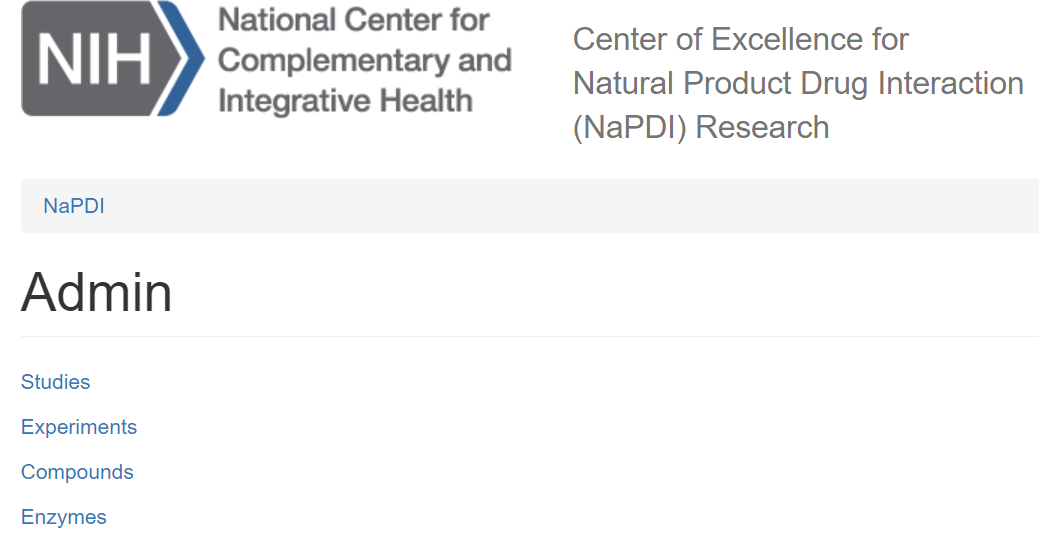
# Creating a study

Use the following steps to create a new study.

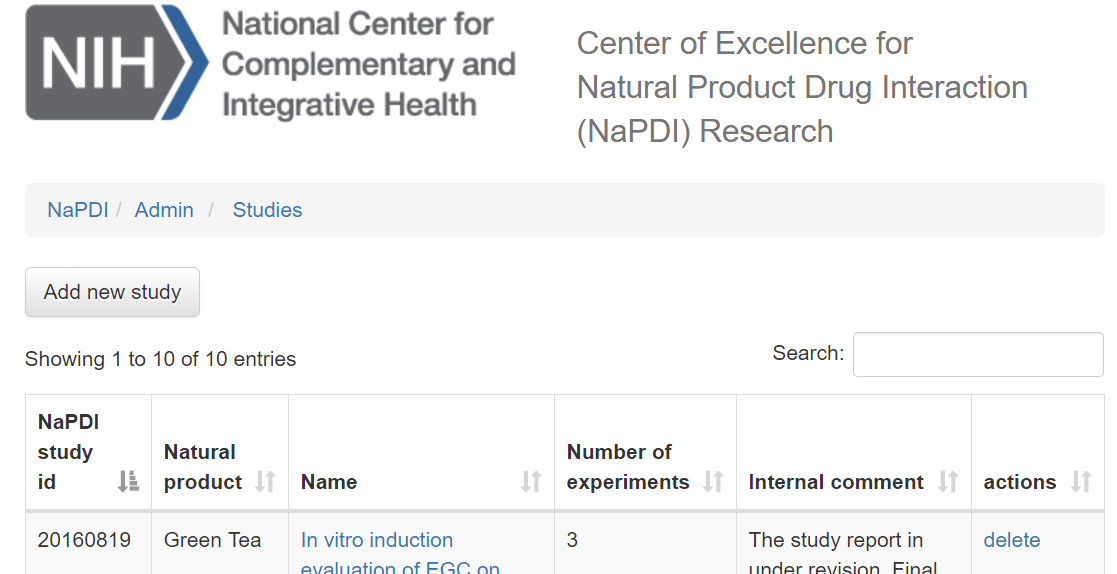
* 1. Navigate to the Admin page of the NaPDI Repository



* 1. Using the admin page, click on “Studies”



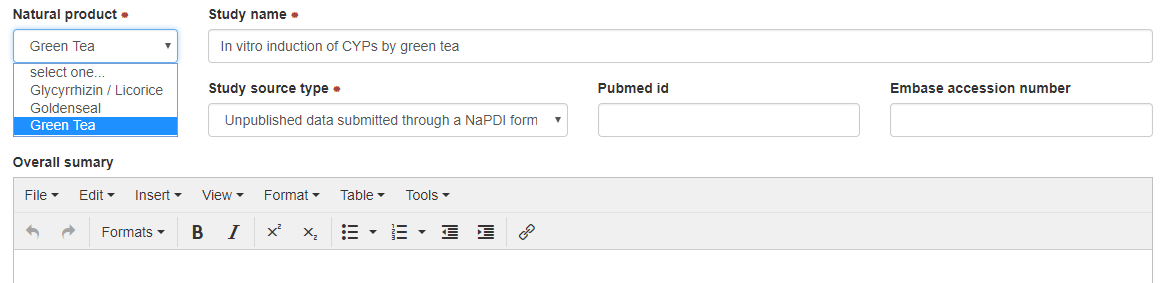
* 1. then, click on “Add new study”



# Study Page

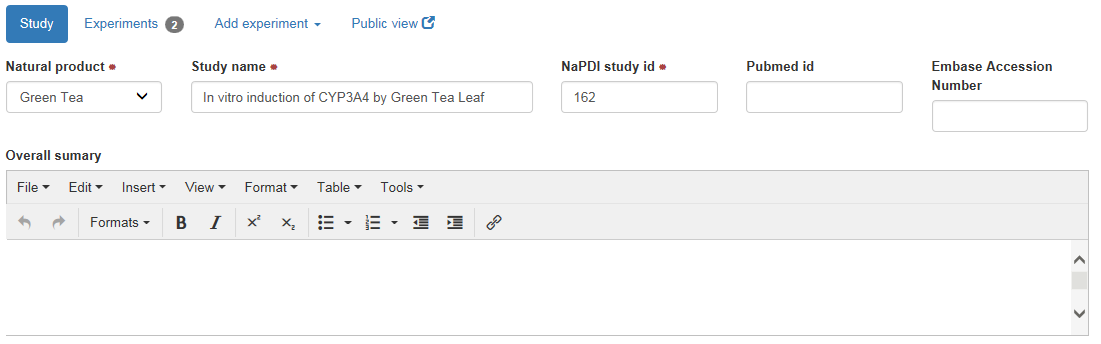
A study can only accept data from one Natural Product and one species. For example, *in vitro* data with Licorice *Glycyrrhiza glabra* L*.*, *Glycyrrhiza uralensis* Fish have to be reported in two different studies, one for each Licorice species.

* 1. Select the **Natural Product** tested in the *in vitro* study from the drop down list provided (select one; required):
* Licorice
* Goldenseal
* Green Tea
  1. From the Study Report, enter the **Study Name** and **NaPDI Study ID** (required, as presented in Study Report).

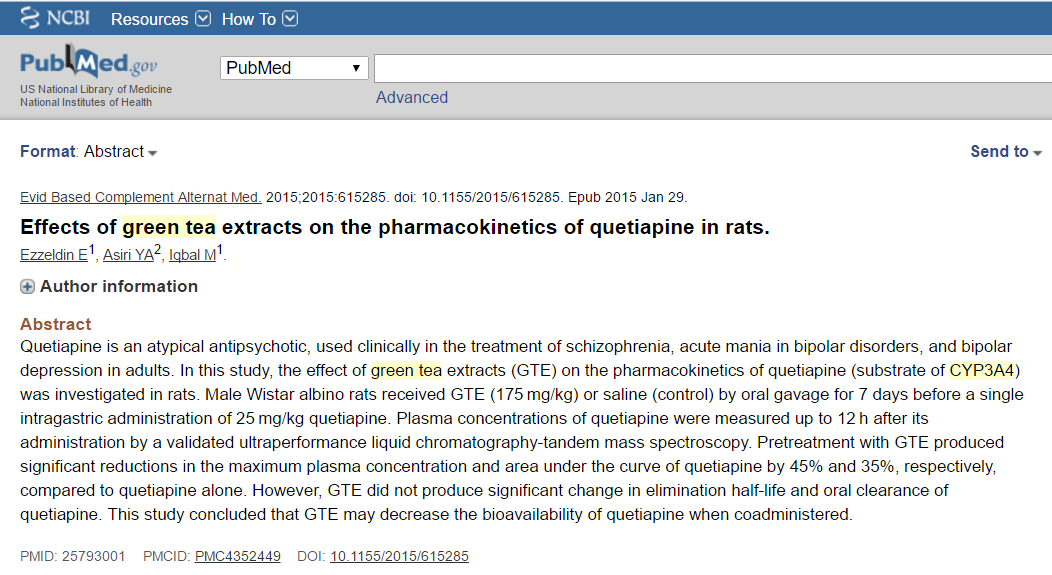


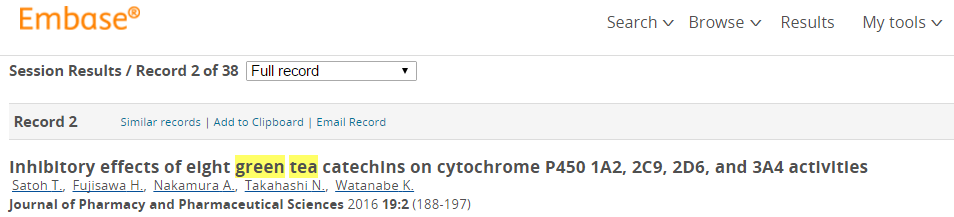
If a entries originate from a published paper, used the Pubmed ID or Embase PUI as the NaPDI Study ID (e.g., “PMID:23268924”)

* 1. Select the **study source type** or source from which the study was obtained (required).
* Published report
* Manuscript prepared or submitted for peer-reviewed publication
* Unpublished data submitted through a NaPDI form
  1. When a study has been published, enter the **PubMed ID** and/or **Embase** **Accession** number(s) (optional)

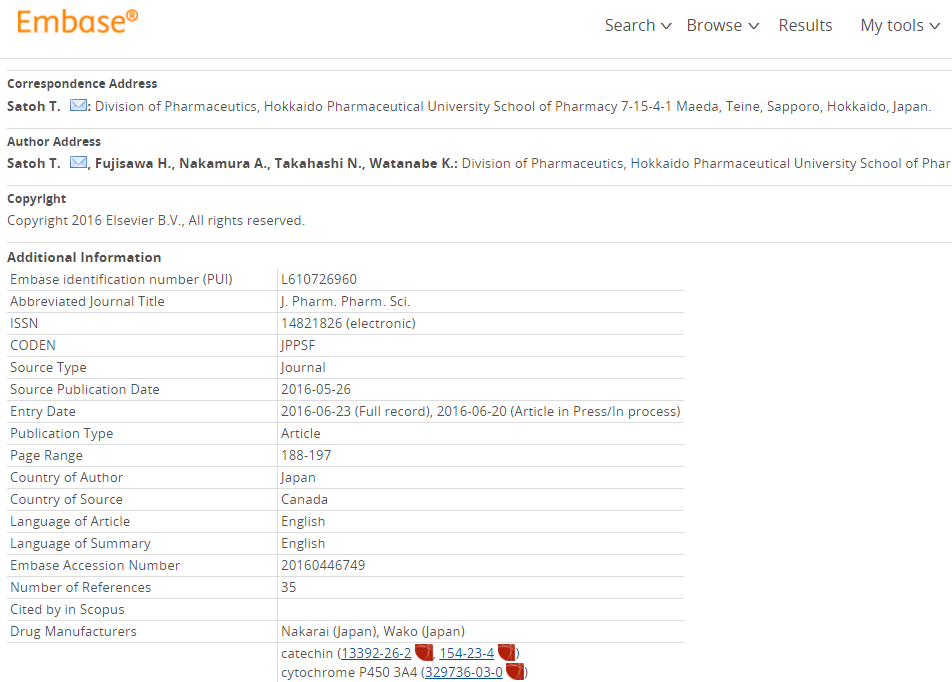


**Tip**: If the PubMed ID or Embase Accession number(s) cannot be located in the Study Report, they can be found under the abstract in PubMed or in the “Additional Information” section when the article’s full record is viewed in Embase.





…



3.4 **Overall summary**: this summary should provide a concise overall conclusion of the *in vitro* study and also discuss the possible mechanism(s) involved (optional).

If entries are from a published paper, copy and paste the abstract into the Overall summary box.

* 1. The **Following for internal use only** section is designated for internal notes and will not be displayed to users.
     1. Enter the **Research organization** name (where the study was performed) and their **study ID number** in the internal use section displayed below (required).
     2. From the Study Report, enter the **dates the study was conducted** (optional). If only months are provided, select the first and last days of the month for the starting and ending date, respectively. For example, March to April, 2017 will be entered as 03/01/2017 to 04/30/2017. **Of**
     3. Enter **internal comments** associated with the study that are intended for internal use only (optional).



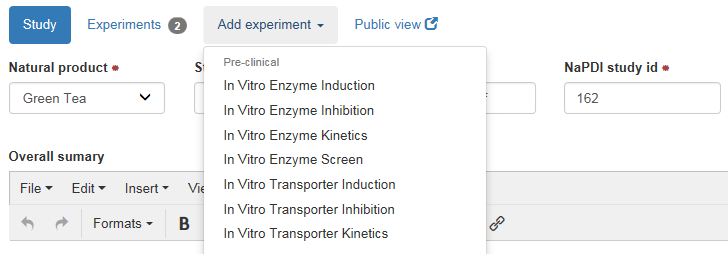
3.6 Select the status of the current study entry

* Draft – selected when the curator is in the process of entering the data or checking the data
* Pending review – selected when the study had been fully entered by the curator and needs to be reviewed and validated by a second editor
* Published – selected after validation and is ready for public display

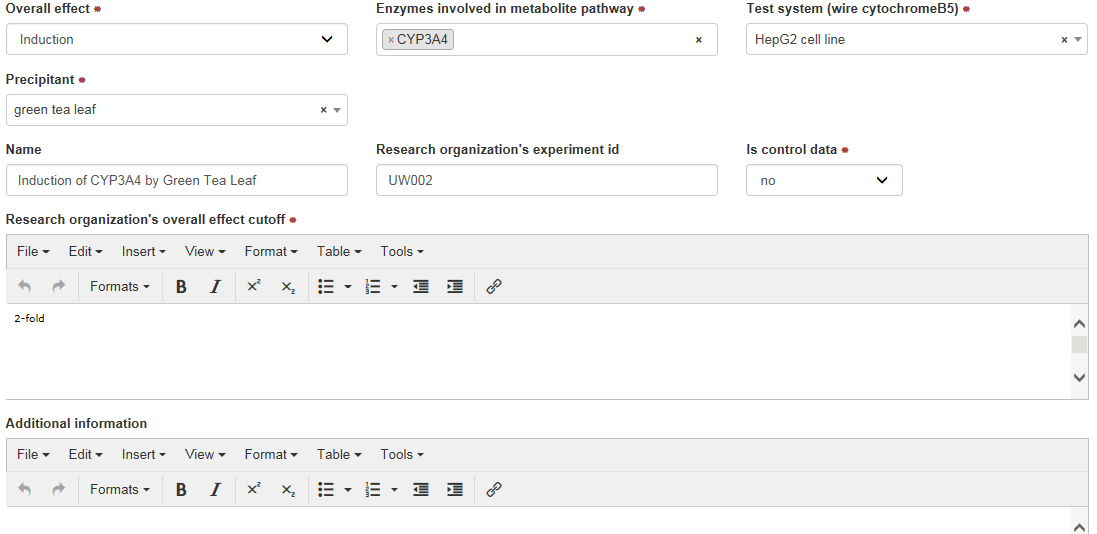
# Experiment

*After* a study has been created, use the following steps to add a new experiment.

* 1. Click on **add experiment**, then select **In Vitro Enzyme Induction** from the drop-down menu.



* 1. Select the **Overall effect**: “Induction”, “Non-induction” or “Down Regulation” of the metabolism of the *Object,* based on study findings stated in the Study Report (select one; required)*.* Use the authors’ conclusions to make this determination. For example, if induction was observed, but the authors concluded no effect because it was not statistically significant, then “Non-induction” should be selected. Conversely, if 1.9-fold inhibition was observed with a 2-fold cut-off and the authors concluded weak induction, then “Induction” should be selected.



* 1. Select the **Enzyme(s)** **involved in the metabolite pathway** as stated in the Study Report (select many; required). Multiple selections can be made; therefore, select all enzymes responsible for the formation of the metabolite (if specific formation of metabolite is measured) or parent disappearance (if parent disappearance is measured).

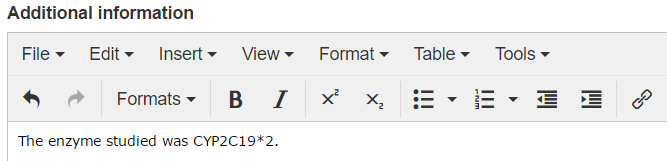
Notes:

1. When an enzyme studied is not listed in the drop-down menu, add the enzyme or state the enzyme in the **Additional information** section (see below).
2. When variant enzymes are studied, select the variant enzyme and specify the variant in the **Additional information** text box (see below).
   1. Select the *in vitro* **test system** used (select one; required).
   2. Select the **Precipitant** from the compound list (select one; required). If the compound is not listed, add the compound.
   3. Select the **Object** and **Object metabolite measured**, if applicable. “Not applicable” may be selected if these fields are not required.
   4. Enter an experiment **Name**, if provided (optional). Use title case, where the first word and all major words are capitalized (i.e., “Induction of CYP3A4 by Green Tea Leaf”, NOT “Inhibition of CYP3A4 by green tea leaf”.) Experiment names are used as sub-headings in the public view; therefore, names that describe the enzyme(s) involved in the pathway of the metabolite and the precipitant used in the study are best suited for this purpose.
   5. If provided, enter the **research organization’s** **experiment identification number** for this experiment only (optional).
   6. If this data corresponds to the control experiment for the study, choose “yes” from the **Is control data** drop-down menu (select one; required). Otherwise, choose “no”. The “Is control data” function allows experiments to be linked within the repository. It only appears on the admin side and not in the public view.
   7. Enter the **Research organization's overall effect cutoff** (required) described in the Study Report (for example, “2-fold versus control”). Enter multiple cut-offs if more than one is provided in the Study Report.
   8. Enter **Additional information** important to the overall study, but where the details were not included in the fields above (optional). Enter only the study vehicle control (i.e. not the positive or negative control) as the object, if the vehicle itself is not specified in the study. Enter the details regarding the positive/negative control in the Additional Information section under this tab.

4.10.1 If the vehicle is specified, enter the vehicle experiment data as a separate experiment, if possible

For example:

* variant enzymes, other enzymes or test systems not listed in the drop-down menu



* variations on the precipitant selected

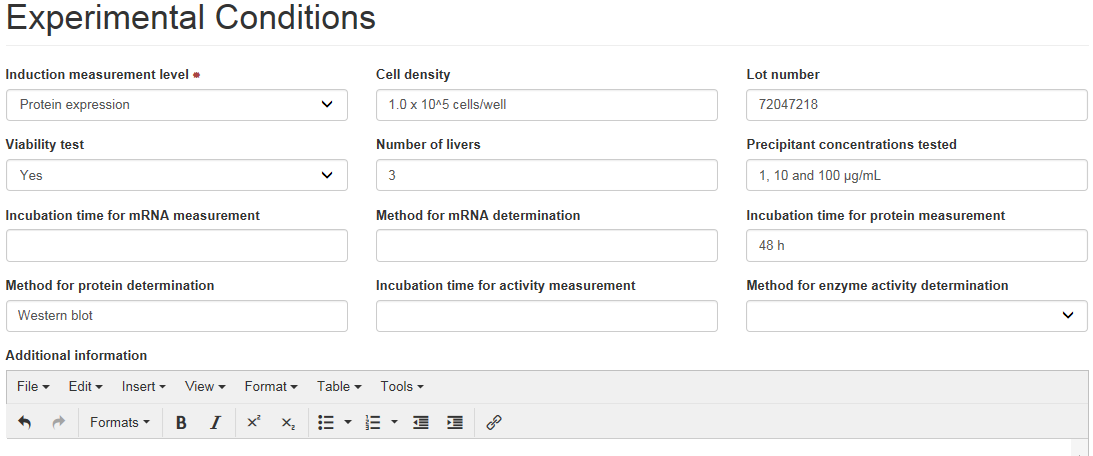


# Experimental Conditions

Enter experimental details that are provided in the Study Report.

Note that in many cases, some of the provided fields may be left blank.

When entering conditions from published literature that refer to experimental conditions described in a reference, check the reference for conditions that are not clearly stated in the article. For example, if an article states that CYP1A2 substrates and concentrations used in Vivid CYP screening assays were used as described in Cheng et al. and the authors do not describe any further details, check Cheng et al. for experimental conditions and enter those stated therein. Also, make a comment in the additional information section regarding which parameters were extracted from the reference citation (*e.g.*, Object and object concentrations tested were extracted from Cheng et al., 2017).



* 1. **Induction measurement level:** Select one of the following from the drop-down menu (select one; required). If results of multiple types of induction measurements are available, enter each as a separate experiment.
* **Enzyme activity**
* **Protein expression**
* **mRNA expression**
  1. **Cell density:** Enter cell density used for the experiment, specify the units as they are presented in the Study Report (optional).



* 1. **Lot number**: If provided, enter the test system lot number for those that were provided by a commercial vendor (optional).



* 1. **Viability Test**: Select “Yes” if a viability test was performed on the cells used in the incubation, select “No” if the test was not performed (select one, optional). Do not make a selection if the information is not provided.
  2. **Number of livers**: Enter the number of liver donors tested (optional).



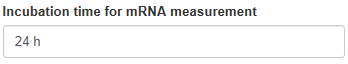
* 1. **Precipitant concentrations tested:** Enter the precipitant concentration(s) used in the incubation, specify the units as they are presented in the Study Report (optional). A single concentration, multiple concentrations or a range of concentrations may be entered.







* 1. **Incubation time for mRNA measurement:**  Use this field only if the “Induction measurement level” selected is “mRNA expression”. Enter the incubation time used for mRNA determination (optional).



* 1. **Method for mRNA measurement:**  Use this field only if the “Induction measurement level” selected is “mRNA expression”. Enter the method used for mRNA determination (optional).



* 1. **Incubation time for protein measurement:**  Use this field only if the “Induction measurement level” selected is “Protein expression”. Enter the incubation time used for protein determination (optional).



* 1. **Method for protein measurement:**  Use this field only if the “Induction measurement level” selected is “Protein expression”. Enter the method used for protein determination (optional).

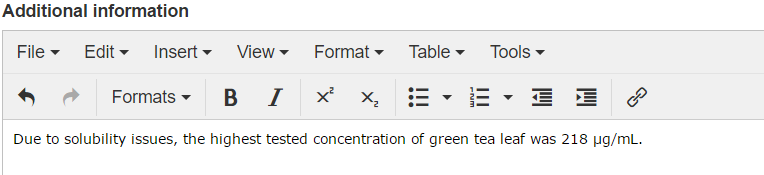


* 1. **Incubation time for activity measurement:**  Use this field only if the “Induction measurement level” selected is “Enzyme activity”. Enter the incubation time used for enzyme activity determination (optional).



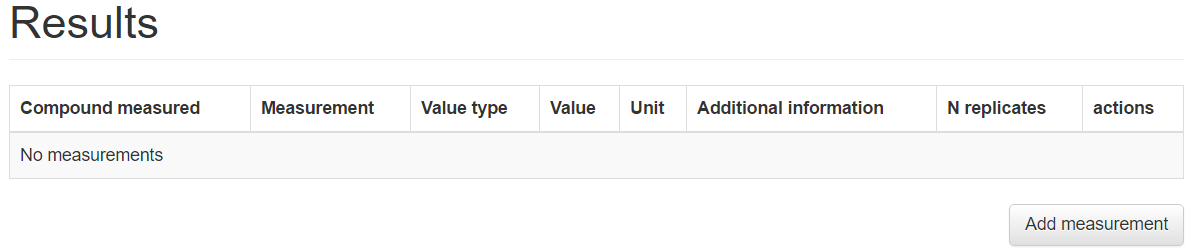
* 1. **Method for enzyme activity determination:**  Use this field only if the “Induction measurement level” selected is “Enzyme activity”. Select the enzyme activity marker used for enzyme activity determination from the drop-down menu (select one, optional).
  2. **Additional information** (optional)**:** As needed, add any other information that is important to the experimental conditions, but that were not detailed in the fields above. In the public view, this section will appear before any of the other experimental details entered.

Examples of additional information might include issues limiting the experimental design (*e.g.*, solubility), deviations from physiological pH (*e.g*., studies were conducted at pH 6.0), etc.



# Results

* 1. Use the **Add measurement** function to add a new measurement to the table of results.



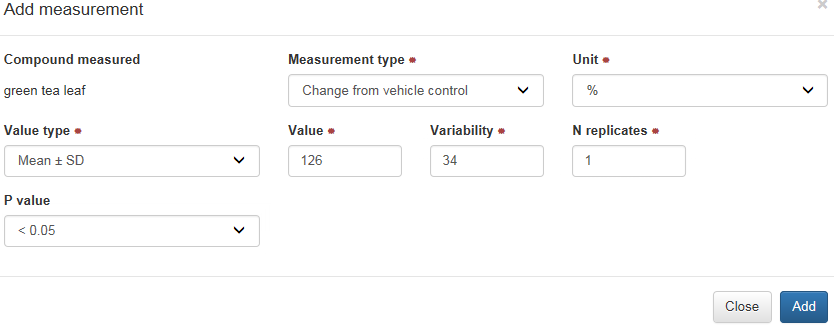
The precipitant compound name selected in the experiment page will automatically be populated in the **Compound measured** field.

* 1. Select a **Measurement type** (select one; required) from the drop-down list, the associated **Unit** (select one; required) and the **Value Type** (select one; required)based on the avaliable data in the Study Report. Use separate entries for each type of measurement.

Note: a common strategy in induction studies is to use cells from at least 3 individual livers in order to capture interindividual variability. In these cases, enter the most potent result. Other results may be entered in the Additional Information section (see below).

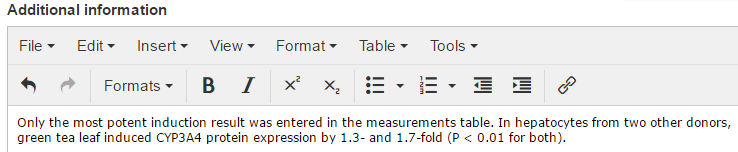
According to the selections, appopriate fields will appear, including the following:

* + 1. **Value** (select one; required)– based on the Study Report choose mean, median, or single value (mean or median is not specified) for the parameter to be entered. Before the value field, select “>”, “≥”, “<”, or “≤” when provided in the Study Report; “=” is the default if no selection is made.
    2. **Variability** (required) –enter the standard error of the mean(**SEM**), percent coefficient of variation (**%CV**), standard deviation (**SD**), 90% or 95% confidence interval (**90% CI**, **95% CI**) or **range** associated with the value.
    3. Enter the total number of **(N) replicates** (required) used in the study (e.g., enter 1 when only one test was conducted, 3 when a total of 3 replicates were used, etc.).
    4. When statistical tests are conducted, select a significance level of the **P value** tested or **not significant** (optional)when the null hypothesis is true. If the authors give a P value that is not avaliable in the drop-down menu, chose the level of significance that is true (e.g., if P = 0.0009 in the Study report, choose P < 0.001).
  1. When all PK measurements have been entered for that entry, click **Add**.



* 1. **Additional Information:** as needed, add any other information that is important to the result, but that were not detailed in the results table.

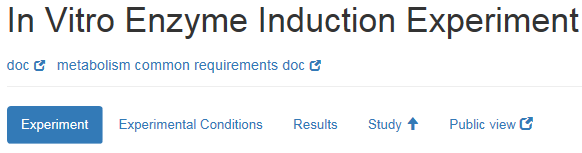
For example,results not entered in the measurements table.





Click to save the entries.

After submitting the study entry, it can be viewed as it will appear to the public by clicking on the “Public View” function near the top of the page.



Note regarding units: For consistency use the following abbreviations for the specified units below. If a unit is not listed below, use the units specified in the Study Report.

|  |  |
| --- | --- |
| Unit | Abbreviation |
| hour(s) | h |
| minute(s) | min |
| second(s) | s |
| day(s) | day(s) |
| liter | L |
| per unit | /unit (*e.g.*, /min) |
| micro | µ |
| fold | -fold (*e.g.*, 3.2-fold) |
| exponents | ^ (*e.g.*, 10^-6) |
| less than, less than or equal to | < , ≤ |
| greater than, greater than or equal to | > , ≥ |
| plus or minus | ± |

* Use molar concentration rather than moles per liter(i.e., use µM rather than µmol/L). In the case of natural products, the use if grams per liter (i.e., µg/mL) may be necessary.
* Do not convert gram concentrations (*e.g.*, µg/mL) to molar concentrations (*e.g.*, µM), even if the molecular weight of the compound is provided.
* If the units provided for a given field are different from the units in its corresponding drop-down menu, convert the units provided in the study report to the units provided in the drop-down menu. If this is not possible (for example, µg/mL cannot be converted to µM for natural product mixtures because there is not a molecular weight available for the conversion), add the new unit to the drop-down menu.