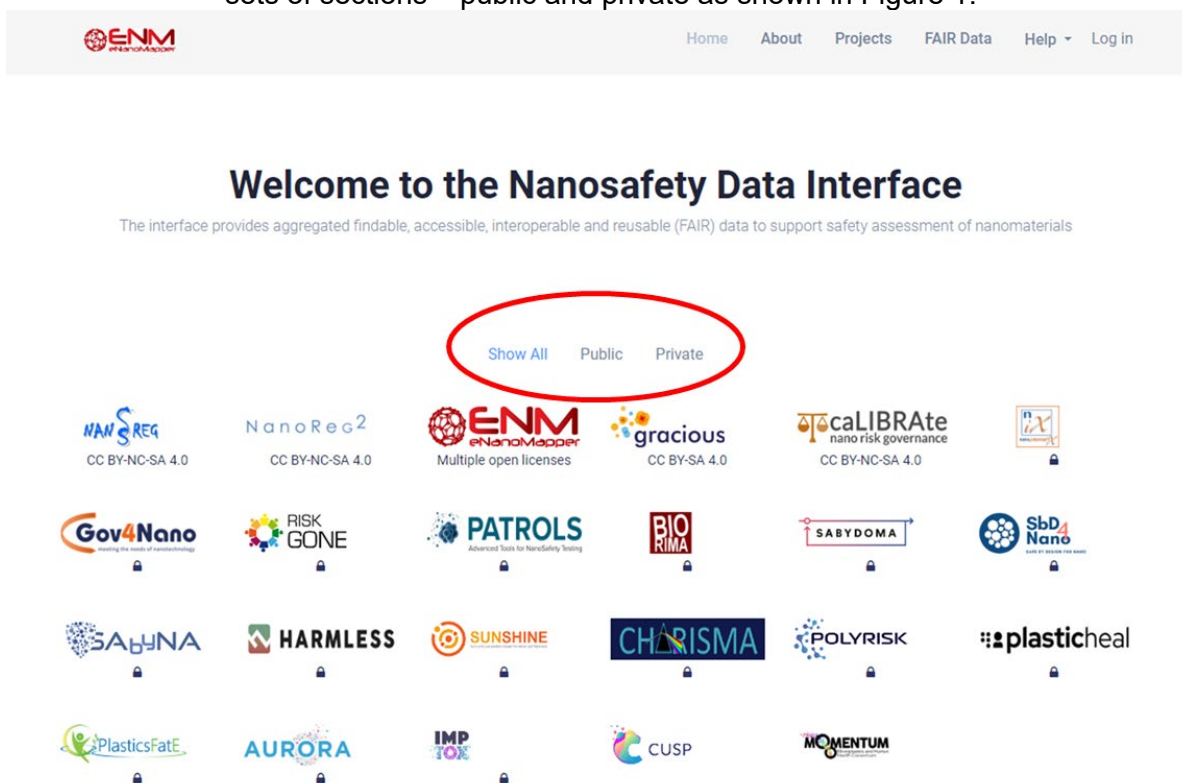


Instructions for use of templates available in the NanoSafety Data Interface

In your internet browser, type or copy <https://search.data.enanomapper.net/>

The NanoSafety Data Interface integrates data from multiple projects, and has two different sets of sections – public and private as shown in Figure 1.



In the “Public database” you can access data with open licenses and the various templates. If you are not a member of an ongoing project, please use the ENM Database, as shown in Figure 2, to customize and download the required templates.

The “Private database” contains data and templates of ongoing projects which are currently restricted to project members. If you are part of an ongoing project listed within the database, please click on the appropriate icon (project logo).

The instruction below are for the public eNanoMapper database, but are also applicable for project databases.

Select the public eNanoMapper database by clicking on the ENM icon as shown in Figure 2.

Welcome to the Nanosafety Data Interface

The interface provides aggregated findable, accessible, interoperable and reusable (FAIR) data to support safety assessment of nanomaterials



Figure 2: Users not associated with a specific running project can select the ENM database which is public.

In the new window that opens, click on Template Wizard to access the templates, as shown in Figure 3. The link will take you to the “**Templates Wizard gallery**”, as shown in Figure 4.

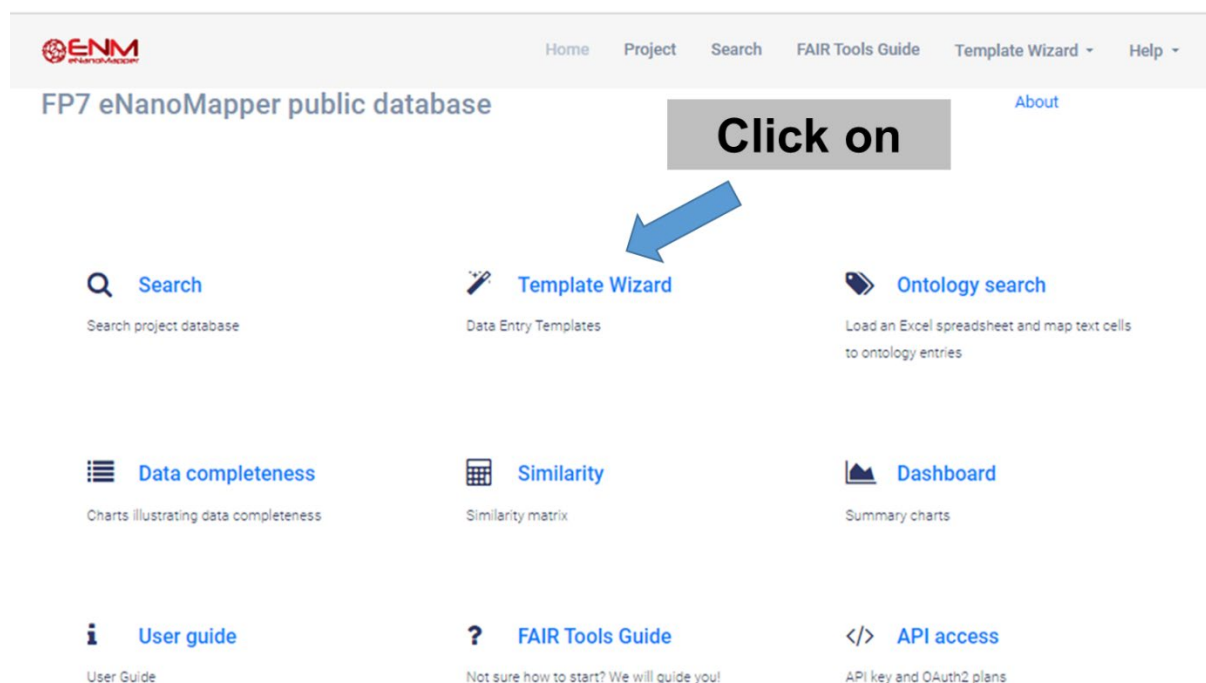


Figure 3. Click on Template Wizard to access the range of templates available – the Template Wizard Gallery (shown in Figure 4).

[Home](#)
[Project](#)
[Search](#)
[FAIR Tools Guide](#)
[Template Wizard](#)
[Help](#)

Templates Wizard gallery

FP7 eNanoMapper public database

Filters Active - 0

Type

Dose response 25

Ecotoxicity 3

Exposure and release 2

Metadata only 3

Phys-chem characterisation 28

Category

Analytical Methods 6

Barrier integrity 1

Bioaccumulation: aquatic / sediment 1

Cell Viability 11

Computational models metadata 1

Search:

Template Wizard link	Template	Project/Provider	Type	Category	Status
ALAMARBLUE	Alamar Blue The Alamar Blue (AB) assay is a high throughput, cell metabolism-based method largely applied in toxicology and nanotoxicology to investigate cell viability (cytotoxicity), cell proliferation and cellular metabolic activity in response to chemicals and nanomaterials.	RISKGONE/NILU	doseresponse	Cell Viability	published
BARRIERCROSSING	Barrier crossing	NANOINFORMATIX/	doseresponse	Barrier integrity	published
BIOACCUMULATION_MUSSELS	Bioaccumulation in mussels	SBD4NANO/INL	ecotox	aquatic /	published

Figure 4: The Template Wizard gallery in the ENM database, in which users can search for templates by type or category via the left and right search boxes, respectively.

Within the two tables above, you can search for templates by “type” or “category.”

For example, if you move the slider under the right hand side “Category” search box you will see all of the available templates.

If you click “cell viability”, a list with 11 template wizards will be shown, as illustrated in Figure 5 below.

The screenshot shows a web application with a sidebar on the right containing a list of categories: Analytical Methods (6), Barrier integrity (1), Bioaccumulation: aquatic / sediment (1), **Cell Viability (11)**, and Computational models metadata (1). The 'Cell Viability' category is highlighted in blue. Below the sidebar is a search bar. The main content area displays a table of templates. A red circle highlights a 'Template Wizard link' above the table. A blue arrow points from a grey box containing the text 'List with cell viability available templates' to the first row of the table, 'ALAMARBLUE'.

Template	Project/Provider	Type	Category	Status
ALAMARBLUE The Alamar Blue (AB) assay is a high throughput, cell metabolism-based method largely applied in toxicology and nanotoxicology to investigate cell viability (cytotoxicity), cell proliferation and cellular metabolic activity in response to chemicals and nanomaterials.	RISKGONE/NILU	doseresponse	Cell Viability	published
BIOIMPEDANCE Label-free Cell monitoring by Electrical Impedance (bioimpedance). This method can assess cell viability, proliferation, cell-cell and cell-substrate interaction of adherent cells growing onto a microelectrode array.	RISKGONE/University of Bergen (UIB)	doseresponse	Cell Viability	published
BIOIMPEDANCE_ECOTOX Label-free Cell monitoring by Electrical Impedance (bioimpedance). This method can assess cell viability, proliferation, cell-cell and cell-substrate interaction of adherent cells growing onto a microelectrode array.	RISKGONE/University of Bergen (UIB)	doseresponse	Cell Viability	published
CARBONYLATION Determining Protein Carbonylation	GRACIOUS/BfR	doseresponse	Cell Viability	published
Colony Forming Efficiency The colony forming efficiency				

Figure 5: The list of currently available (publicly) templates or in vitro dose-response studies, including the Alamar Blue cytotoxicity assay which is used for the subsequent illustration of the

The next steps are illustrated for an in vitro dose-response assay, but are similar for any selected template.

Click on the name of the specific (cell viability) assay that you have performed. Let's use **the Alamar Blue (AB) assay**, for example.

A new window will open, as shown in Figure 6.

Before downloading the template, you must fill in all positions marked with red.

Then click on the blue **Download template** button (at the top right in Figure 6), and save the file (test data recording template, TDRF).

ENM Home Project Search FAIR Tools Guide Template Wizard Help

Template Wizard : in-vitro assays

FP7 eNanoMapper public database

Choose assay: **Alamar Blue** Excel template download **Download template**

SOP: **HEL16T008 AlamarBlue Assay** Enter medium: **DMEM + 10% FBS** Enter type of plate: **96-well**

Enter cell name: **A549** Enter passage No: **4** Enter cells per well: **10000** Enter volume per well: **0.2 ml** Experiments: **3** Replicates: **2**

Enter concentrations: **0,1,2,5,10,25,50,100,SC,PC,NC2,INT_1,INT_10C** Enter unit: **ug/cm2** Enter number of readings: **4**

Enter time points: **24** Enter unit: **h** Enter negative control(s): **0,NC2,SC** Enter positive control(s): **PC**

Project: **ENANOMAPPER** Partner/organisation: **Partner** Website: **WP**

Materials table - all materials will be available for selection in the Excel file

Show 10 entries

ERM	ID	Name	Type	Supplier	Supplier code	Batch	Core chemistry	CAS	BET surface (m ² /g)
NM-110	NM-110	NM-110		JRC			ZnO	1314-13-2	
NM-200	NM-200	NM-200		JRC			SiO2	7631-86-9	

When opening the saved file in Excel, you will see the following (Figure 7):

TestDataRecordingForm_enanomapper_ALAMARBLUE - Excel

File Home Insert Page Layout Formulas Data Review View Tell me what you want to do...

Spelling Thesaurus Smart Lookup Translate New Comment Delete Previous Next Show/Hide Comment Show All Comments Show Link Protect Sheet Protect Workbook Workbook Changes

V31

Enanomapper **IN-VITRO Test Data Recording Form (IDRF)**

1. Please complete all applicable fields below as far as possible. Aim to familiarise yourself with the Introductory Guidance and Example Filled Templates.

2. While aiming to standardise data recording as far as we can, flexibility may still be needed for some TestAssay types and their results.

3. Thus it may be necessary to add additional items e.g. for further replicates, concentrations, timepoints, or other variations on inputs, results outputs, etc.

4. If you please highlight changes & alterations e.g. using colour, and/or comments in notes, or adjacent to data tables to flag items, fluctuations from norm, etc.

5. Please ensure you also complete a Test Method Description-Form (TMDP) for this test type.

IN-VITRO TEST CONDITIONS

Project Work Packages: Select from project drop-down list

Partner conducting testassay: Select from partner drop-down list

Test facility - Laboratory name: Enter text

Lead Scientist & contact for test: Enter text

Assay/test work conducted by: Enter text

Full name of testassay (add OECD Test ref. ID if app.): Enter text

Short name or acronym for testassay: Enter text

Type or class of experimental test as used here: Enter text

End-Point being investigated/assessed by the test: Enter text

Metric(s) used to assess End-Point outcome/responder: Enter text

SOP(s) for test - ref. project or other doc. - Time/Ref: Enter text

Link to SOP/protocol on proj. server/web where applicable: Enter text

Test start date (YYYY-MM-DD): 2023-06

Test end date (YYYY-MM-DD): 2023-06

Enter full test substance details in this block

Select item from Project Materials list

Material Name: E.g. SiO2 (silicon dioxide), TiO2 (titanium dioxide), etc

Material Supplier: Enter text

Material Status: Enter text

Batch: Enter text

Material Supplier: Enter text

Material Status: Enter text

Batch: Enter text

Date of preparation (YYYY-MM-DD): 2023-06

Endotoxin confirmed as absent?: Enter text

DISPERSION

Specify standard dispersion protocol used: Enter text

Or otherwise specify dispersion technique used: Enter text

Additives used? If yes, specify which & conc. used: Enter text

Dispersed in cell culture medium?: Enter text

Aids used to disperse: Enter text

Specify time-duration: Enter text

Energy (for sonication): Enter text

CELL LINE DETAILS & CULTURE CONDITIONS

Detailed cell type/line specification: Enter text

Cell line short name: A549

Supplier: Enter text

Passage no: 4

Plate details as applicable: 96-well

Number of readings (Absorbance): 4

Number of cells per well: 10000

Total volume per well: 0.2 ml

Medium (Supplier/Lot No.): DMEM + 10% FBS

Serum (inc. supplier/Lot No.): Enter text

Serum concentration in culture medium: Enter text

Serum concentration in treatment medium: Enter text

Was serum heat inactivated? If app.: Enter text

TREATMENT TIMELINE

Please specify units e.g. Enter text

Test conditions Raw_data_ALAMARBLUE Results_ALAMARBLUE Materials

Figure 7: The test data recording template, after downloading the selected template from the ENM template Wizard.

Under the “Review” window, deselect “Show all comments,” and the explanations will be deactivated. They can be re-activated with the same procedure. These comments help you by describing how to fill the necessary information in the rows (marked in yellow), in case you are not sure what to write.

In the Excel file, you must fill data in the “Test conditions” and “Raw_data_ALAMARBLUE” sheets. Please, look at the “Materials” sheet (by moving between Tabs on the bottom left of the Excel file) and if needed, add the information about the nanoparticles or nanomaterials used. We note that the same templates can be utilised for other materials (e.g., micro or nanoscale plastics) or chemicals in general.

In the “Test condition sheet”, please fill in all necessary information in the rows highlighted with yellow and type the name of the project you are working on, as shown in Figures 8 and 9 below. This information forms part of the metadata that accompanies the data and makes the data more findable once it is uploaded to the eNanoMapper or other database.

IN-VITRO TEST CONDITIONS

Project Work Package: WP2
 Partner conducting test/assay: IB-BAS
 Test facility - Laboratory name: LMBI
 Lead Scientist & contact for test: A. Bala
 Assay/Test work conducted by: Ani Vega
 Full name of test/assay (add OECD Test ref ID if app.): ALAMARBLUE
 Short name or acronym for test/assay: ALAMARBLUE
 Type or class of experimental test as used here: generic
 End-Point being investigated/assessed by the test: Relative cell viability
 Metric(s) used to assess End-Point outcome/response: Fluorescence
 SOP(s) for test - ref. project or other doc. - Title/ID: HEL16T008 AlamarBlue Assay
 Link to sop/protocol on proj. server/web where applic.: https://www.rivm.nl/sites/default/files/2016-11/NAtoREC%2005.07%20SOP%2006%20HEL16T008%20AlamarBlue%20Assay.pdf

TEST MATERIAL DETAILS

Select item from Project Materials list: NM-110
 Material Name: NM-110
 NM Core chemistry: ZnO
 CAS No: 1314-13-2
 Material Supplier: JRC
 Material State: powder
 Batch: 111-112
 Date of preparation (YYYY-MM-DD): 2023-06-14
 Endotoxin confirmed as absent?: Pending

DISPERSION

Specify standard dispersion protocol used: https://www.anses.fr/en/system/files/nanogenotox_deliverable_5.pdf
 Or otherwise specify dispersion technique used:
 Dispersion agent?: BSA-water
 Additives used? If yes, specify which & conc. used: 30 ul, 0.5 vol% EtOH (96% or higher) is used for pre-wetting the NPs
 Dispersed in cell culture medium?: no
 Aids used to disperse:
 Sonication-Bath: Branson Sonifier S-450D
 Sonication-tip: 13 mm disruptor horn
 Vortexings: no
 Stirring: no

CELL LINE DETAILS & CULTURE CONDITIONS

Detailed cell type/line specification: https://www.atcc.org/products/cccl-185
 Cell line short-name: A549
 Supplier: ATCC
 Passage no: 4
 Plate details as applic.: 96-well
 Number of readings (Absorbance): 4
 Number of cells per well: 10000
 Total volume per well: 0.2 ml
 Medium (Supplier/Lot No.): DMEM, high glucose, HEPES, no phenol red
 Serum (inc. supplier/Lot No.): ThermoFisher Scientific, 11X-S11111
 Serum concentration in culture medium: 10% FBS

Figure 8: The “Test condition sheet” allows the user to add the study conditions (cells highlighted in yellow in the template), which form part of the metadata (data about the data). The project name allows data to be aggregated with other data from the same project, or for users not aligned with a specific research project acts as an identifier to facilitate data search.

Figure 9: Continuation of the **Test condition sheet**” allows the user to add the study conditions (cells highlighted in yellow in the template).

Now your template is ready for use to capture your experimental data. Once you have performed your experiment, please fill in the raw data obtained for the AB assay in your Lab into the sheet “**Raw_data_ALAMARBLUE**”, shown in Figure 10.

Figure 10: Results sheet ready for the experimental data to be captured. The replicates, concentrations, timepoints etc. **are exactly as was specified** in the **Test Condition Sheet** in the previous step. The automatic calculations including average and normalisation formulae are coded into the Excel file also, reducing the risk of error in calculation.

All results will be automatically calculated and shown in the “**Results_ALAMARBLUE**” sheet.