

MSInspector - QA targeted MS data for CPTAC assay portal

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This tutorial will guide you through the installation of MSInspector and subsequently guide you to perform Quality Assurance (QA) on a sample Skyline file. The underlying process uses Python programming scripts and related Python modules which all will be automatically installed as part of MSInspector external tool installation. Meanwhile, considering that R scripts are called for data visualization and statistical calculation, the executable R file, "Rscript.exe," should be downloaded and installed (R v3.5.2 is recommended).

In order to use MSInspector with Skyline, it must be installed through the Skyline external tools framework. Furthermore, MSInspector requires the use of custom annotations in Skyline based on the predefined Skyline template for individual experiment.

This tutorial covers the installation of MSInspector and how to run the tool itself.

Getting Started

To start this tutorial, download MSInspector_tutorial.zip.

Extract the files to a folder on your computer. This will create a new folder: MSInspector_tutorial

The zip file contains the following file:

MSInspector_ResponseCurve_test.sky.zip: Contains a Skyline document with sample data of Experiment 1 for MSInspector.

MSInspector_Repeatability_test.sky.zip: Contains a Skyline document with sample data of Experiment 2 for MSInspector.

You will use the dataset as an example. To open this dataset perform the following steps:

- Open Skyline.
- On the **File** menu, click **Open**.
- Change the **file type** in the **File name** field from 'Skyline Documents (*.sky)' to 'Shared Files (*.zip)'
- Navigate to the *.sky.zip file in the folder you created and open it.
- Alternatively, you can also extract the zip file first and then open the *.sky file directly.

Installing MSInspector as an External Tool

The Skyline external tools framework supports the direct integration of statistical analysis tools that process the data in a Skyline document.

To add MSInspector as an external tool, perform the following steps:

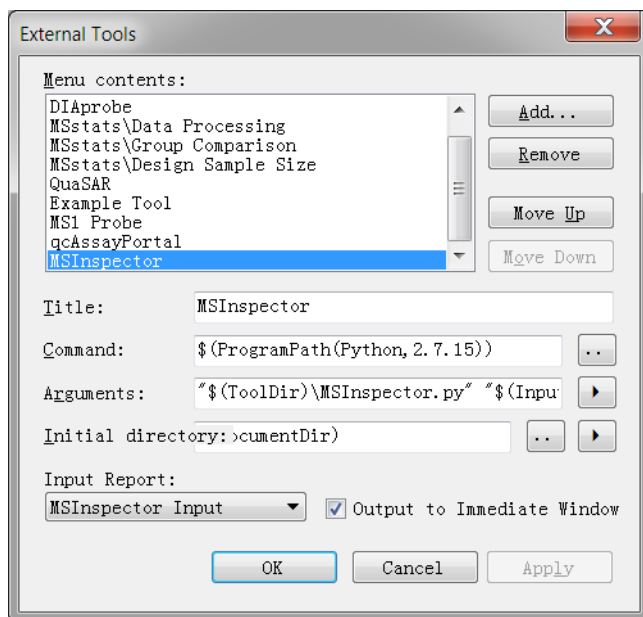
- On the **Tools** menu, click **External Tools** to bring up the **External Tools** form.

- Click the **Add** button, and select **From File...** from the menu that appears.
- Navigate to the location of the MSInspector_1.1.zip file in the folder you created and select it.
- Click **Open**.

Skyline will now guide you through the process of installing MSInspector as an external tool. This process may require you to install the Python programming environment (currently Python 2.7, 32-bit will be installed even if a 64-bit computer is used due to Python compatibility aspects), as well as a number of Python extension packages, including 'pandas' and 'Jinja2'.

- If you do not have the specified version of Python 2.7 installed on your machine, wait for Python 2.7.15 to download. When the download is completed, you will be asked to install Python onto your computer. Complete the Python installation process by working through the Python installer wizard.
- Once the Python installation is completed or Python was initially already found in the computer registry, Skyline will look for the Python package manager Pip on the user's machine which was installed with Python. Skyline installs zip or tar.gz packages of 'pandas' and 'Jinja2' using pip install <package>.

Once the installation is complete, the MSInspector tool will appear in the **External Tools** form:



- If the computer has a preinstalled Python2.7 (version < 2.7.15 is allowed), check whether pip is installed or not. If pip is not installed, please install it by referring to <https://pip.pypa.io/en/stable/installing/>. Then check whether the packages of pandas and Jinja2 are installed or not by running "pip freeze". If they are not installed, run "pip install pandas==0.21.0 Jinja2==2.9.6" to install them. Then check the version of the package of numpy by running "pip show numpy". If the version is lower than 1.13.0, update numpy by running "pip install numpy --upgrade". If you come across error message "Microsoft Visual C++ 9.0 is required" when installing the Python packages, please install Microsoft Visual C++ Compiler for Python 2.7 from

<https://www.microsoft.com/en-us/download/details.aspx?id=44266>

Finally, Click .. button on the right of **Command** label and choose **Edit Macro...** to set the path of Python.

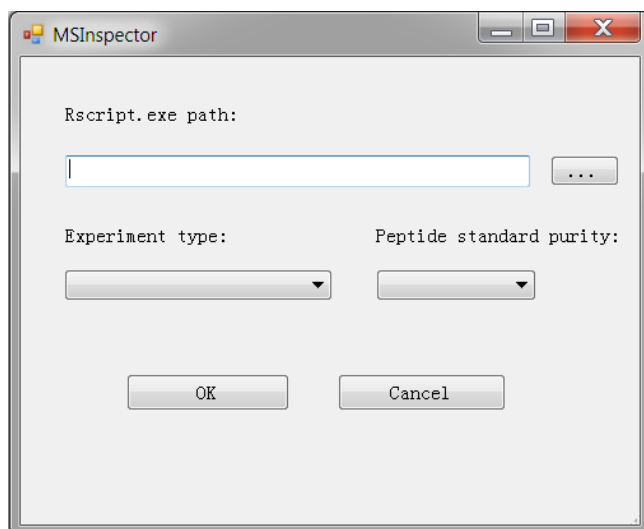
- Click to **OK** button on the **External Tools** form.

The built-in R scripts are called for data visualization and statistical calculation. First, download and install R v3.5.2 from: <https://cran.r-project.org/bin/windows/base/old/3.5.2/R-3.5.2-win.exe>
The executable R file, "Rscript.exe," could be found in the default directory – "C:\Program Files\R\R-3.5.2\bin\Rscript.exe". Then install the following packages in R console by running:
`install.packages(c("Cairo", "evaluate", "reshape2", "stringr", "plyr", "MASS", "ggplot2", "dplyr"))`

Using the **MSInspector** Tool to generate a QA report

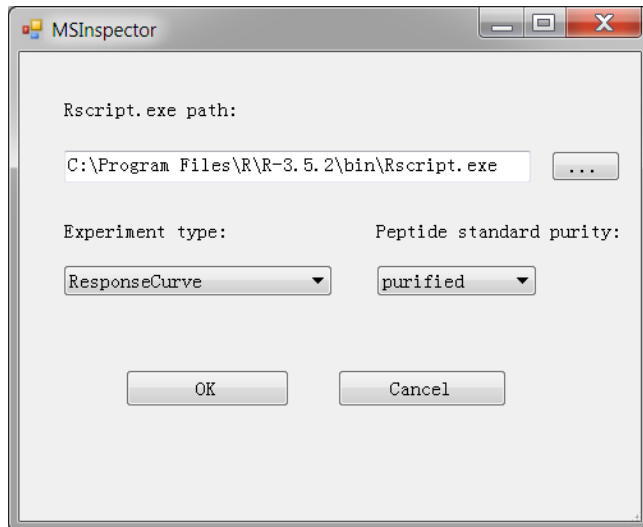
- Annotate the data according to the Skyline templates based on the experiment type. (For the details, please refer to Assay Portal User Manual)
- In Skyline, from the **Tools** Menu, select the **MSInspector** option.

The following **MSInspector** form will appear:

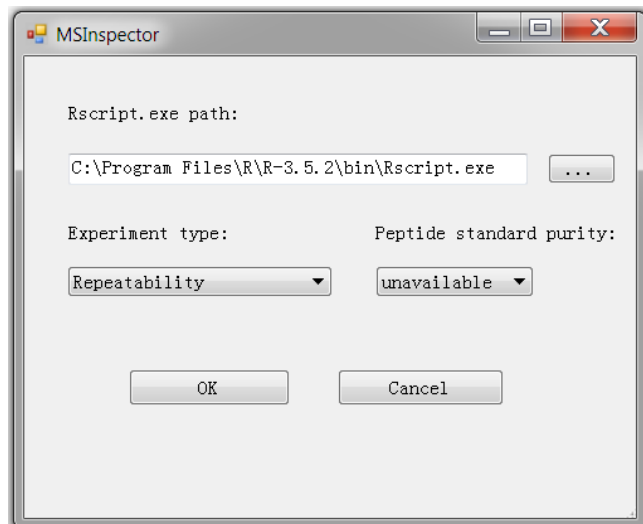


- For **Rscript.exe path** navigate to the location of Rscript.exe.
- Choose an option in **Experiment type**.
- Choose an option in **Peptide standard purity**.

If you open MSInspector_ResponseCurve_test.sky.zip, your MSInspector form should look as follows:



If you open MSInspector_Repeatability_test.sky.zip, your MSInspector form should look as follows:



- Click the **OK** button.

The MSInspector tool will now perform QA. The output from the tool run is shown in the **Immediate Window**, the textbox docked at the bottom of the main Skyline window. When the tool run is completed, switch to the newly created subdirectory named MSInspector_ResponseCurve_test and MSInspector_Repeatability_test. The QA report is generated there.

In the subdirectory of MSInspector_ResponseCurve_test, the *.html file should look as follows:

MSInspector Report

Summary

- Targeted MS Assays Information
- Quality of Internal Standard Type
- Peptides With Errors
- Peptides With Warnings
- Peptides Without Issues

Targeted MS Assays Information

Skyline File Name	Experiment Type	Internal Standard Type	Proteins	Peptides	Precursors	Peptides With Errors	Peptides With Warnings	Peptides Without Issues
MSInspector_ResponseCurve_test.sky	Response Curve	light	8	8	16	1	4	3

Quality of Internal Standard Type

Skyline File Name	Internal Standard Type	Quality
MSInspector_ResponseCurve_test.sky	light	Correct

Peptides With Errors

Skyline File Name	Peptide sequence with errors	Issue reason
MSInspector_ResponseCurve_test.sky	ETPAASEAPSSAAK	* More than one area values exist for the combination of protein, peptidomodsifdsequence, precursorchange, productcharge, fragmentation, replicate, concentration, samplegroup, isspike and isotopelabel.

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In the subdirectory of MSInspector_Repeatability_test, the *.html file should look as follows:

MSInspector Report

Summary

- Targeted MS Assays Information
- Quality of Internal Standard Type
- Peptides With Errors
- Peptides With Warnings
- Peptides Without Issues

Targeted MS Assays Information

Skyline File Name	Experiment Type	Internal Standard Type	Proteins	Peptides	Precursors	Peptides With Errors	Peptides With Warnings	Peptides Without Issues
MSInspector_Repeatability_test.sky	Repeatability	light	3	3	8	0	3	0

Quality of Internal Standard Type

Skyline File Name	Internal Standard Type	Quality
MSInspector_Repeatability_test.sky	light	Correct

Peptides With Errors

Peptides With Warnings

MSInspector_Repeatability_test.sky

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For the details of the issue (including error and warning) messages and the solutions to the issues, please refer to the document named **issue_categories.pdf**.

The work flow of MSInspector is shown in **workflow.pdf**.