

Agilent MassHunter DAR Calculator Software

Quick Start Guide

For Research Use Only. Not for use in diagnostic procedures.

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Where to find more information

- *Online Help* provides in-depth information and can be displayed in the following ways:
 - Click **Help** from the **File** menu.
 - Press the **F1** key to get more information about a window or dialog box.



Agilent Technologies

What is Agilent MassHunter DAR Calculator Software?

Agilent MassHunter DAR Calculator software is a stand-alone program specifically designed to investigate drug-to-antibody ratios (DAR) of deconvoluted LC/MS sample data acquired from antibody-drug conjugates (ADC). The DAR Calculator allows you to interactively view the deconvoluted ADC spectrum, interactively and numerically input mass values for the unconjugated antibody and the drug/linker (the drug molecule and linker complex), adjust peak calculation parameters, and generate DAR calculation results. DAR Calculator reports may be saved as PDF and DOCX files, and your DAR Calculator investigation can be saved as a DAR file and in a .D MassHunter data file.

DAR Calculator provides you with the drug-to-antibody ratios for your sample through a sequential set of operations: for intact ADCs and for reduced ADCs.

Automated DAR Calculator Workflow

You can automatically calculate DAR when you create a method with a custom workflow in the Agilent MassHunter BioConfirm program. You can then run this BioConfirm method from either the Agilent MassHunter Workstation Data Acquisition program or from the Agilent MassHunter Walkup software. See [“Automated DAR Calculator Workflow”](#) on page 17 and [“To create and run a Data Acquisition worklist”](#) on page 29.

What's New in B.01.01

- DAR Calculations can be run from the BioConfirm program.
- DAR specific columns in MassHunter Data Acquisition and MassHunter Walkup allow for automated DAR calculations.

Agilent MassHunter DAR Calculator Software Installation

To install the software

BioConfirm B.08.00 or later must be installed before you install DAR Calculator software.

If the G4994AA Agilent MassHunter DAR Calculator Software is not already installed on your system, install it as follows.

- 1** Insert the disk labeled **Agilent Technologies ADC DAR Calculator Software** into the disk drive.
- 2** From the installation disk, right-click **Setup.exe**, and then select **Run as administrator**.
- 3** Follow the instructions on the screen to install the software.

For Excel based reporting to work properly in the BioConfirm program, after installing Excel 2013 or Excel 2016, you need to open Excel and click **Accept** on the “First things first” message.

To remove the software

Use **Programs and Features** in Windows Control Panel to remove Agilent MassHunter DAR Calculator Software.

User Interface

Main Functional Areas

The main BioConfirm window consists of three parts: (1) the Menu Bar, (2) the Ribbon, and (3) the Main Window. The main functional areas are shown in Figure 1.

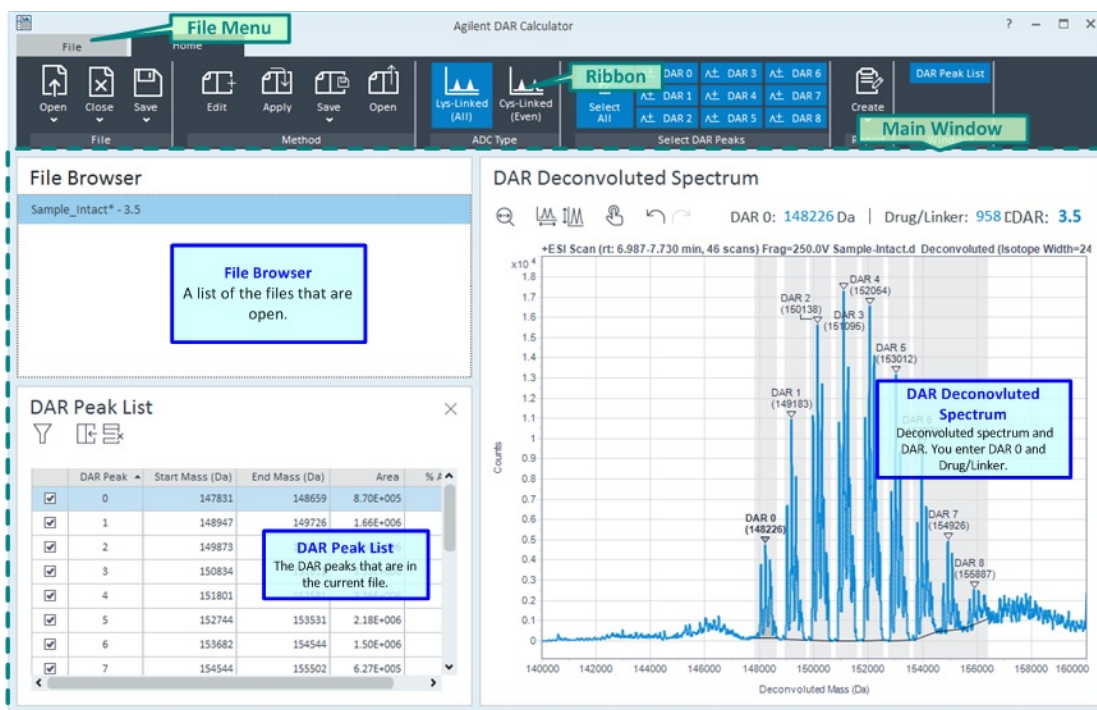


Figure 1 The main functional areas of DAR Calculator

1. File Menu

The File Menu (Figure 2 on page 5) is shown when you click on the File tab in the Ribbon. You can open the **Properties** dialog box, open the Help file, find version information, and lock the program from the **File** menu.

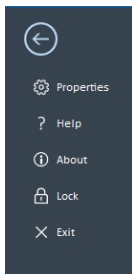
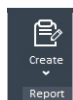


Figure 2 File Menu

2. Ribbon

The Ribbon has two parts: the File Menu, and the Home tab. The Home tab has six groups.

Toolbar Icon	Action
	<ul style="list-style-type: none"> Open an Intact CSV file, one or two Reduced CSV files, or a data file Close one or all open files Save, Save As, or Save All results
	<ul style="list-style-type: none"> Opens the Edit DAR Method dialog box Applies the method that you select to the current file Saves or Save as the method Open a different method to edit
	<ul style="list-style-type: none"> Lys-Linked (All) can have any number of drug molecules attached to the antibody. Cys-Linked (Even) only has even numbered DAR peaks available.
	<ul style="list-style-type: none"> Shows the DAR peaks that are contributing to the DAR calculation.



- Creates a report and saves it as either a PDF or a Word document.



- Toggles whether or not the DAR Peak List pane is visible

3. Main window

The main window, see [Figure 1](#) on page 4, is further divided into three panes: File Browser pane, DAR Deconvoluted Spectrum pane, and DAR Peak List pane. The File Browser pane and the DAR Deconvoluted Spectrum pane are always visible.

File Browser pane This pane contains a list of the files that are open. It shows the file names and the DAR value, if it has been calculated. When you click a different file in the list, the information in the DAR Deconvoluted Spectrum pane and the DAR Peak List pane are updated.

You cannot close this pane.



Figure 3 File Browser pane

DAR Deconvoluted Spectrum You display deconvoluted spectra in this window. In order to determine the DAR value, you enter the DAR 0 value and the Drug/Linker value in the toolbar for this pane. The DAR peaks that are included in the calculation of DAR are highlighted with vertical gray bars. You can remove DAR peaks from the calculation using the Ribbon, the DAR Peak List pane, or the shortcut menu.

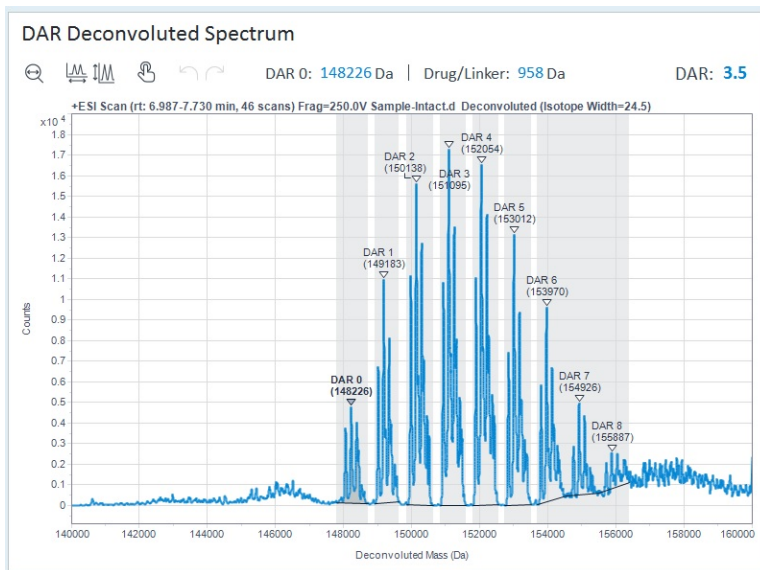


Figure 4 DAR Deconvoluted Spectrum pane

DAR Peak List pane This pane has two parts: a toolbar and a table. The table shows information about each DAR peak in the deconvoluted spectrum. If you delete a peak from the DAR Deconvoluted Spectrum, it is also removed from this table. You can clear the check box at the beginning of a row in the table to remove that peak from the calculation of DAR. By the shortcut menu, you can export the table, filter selected peaks, delete peaks, and edit the method.

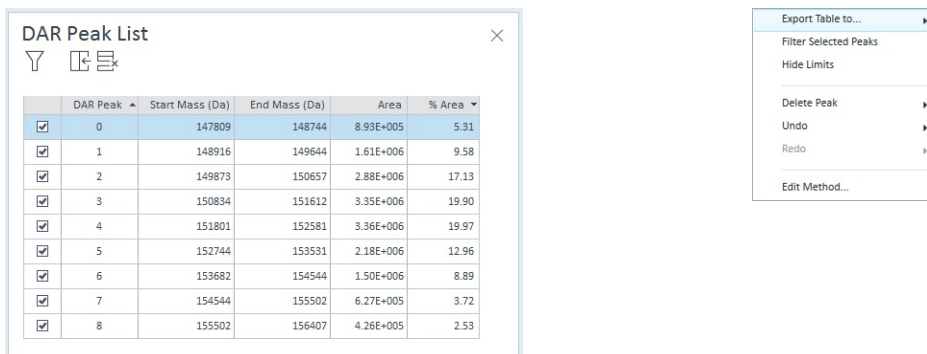


Figure 5 DAR Peak List pane and shortcut menu

DAR Dialog Boxes

The following dialog boxes are some of the dialog boxes available in Agilent MassHunter DAR Calculator Software. See *online Help* for more information.

Edit DAR Properties Dialog Box

This dialog box allows you to set global parameters that are used when you open a new CSV file.

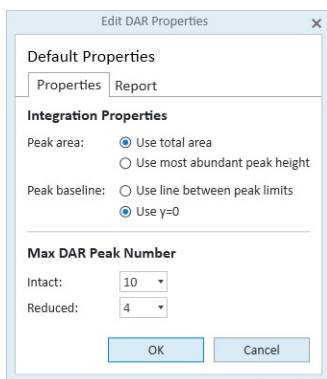


Figure 6 Edit DAR Properties dialog box

To open this dialog box Click **Properties** in the File menu.

Edit DAR Method Dialog Box

You can edit a method and save the settings that you want to apply to the deconvoluted spectrum. If you are processing similar data files, then you can apply a method instead of manually entering information when you open a different file.

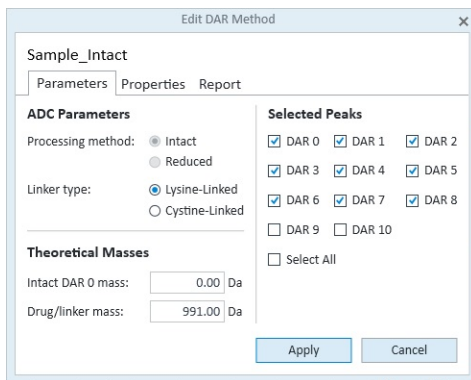



Figure 7 Edit DAR Method dialog box

To open this dialog box Click  in the Method group in the Ribbon. You can instead click **Edit Method** in the File Browser shortcut menu.

Select method to apply Dialog Box

You can select any method that was saved, and this method is applied to the current file.

To open this dialog box Click  in the Method group in the Ribbon.

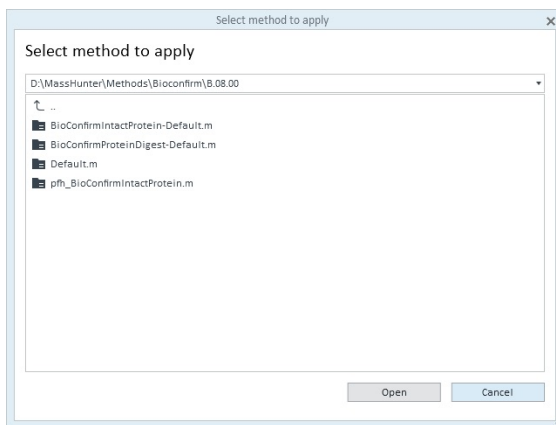


Figure 8 Select method to apply Dialog Box

Basic Tasks

To start the DAR Calculator program

Basic Tasks

Basic DAR Workflow

“To start the DAR Calculator program” on page 10

“To create CSV files in BioConfirm” on page 10

“To process Intact CSV file” on page 12


“To process Reduced CSV files” on page 13

“To edit results” on page 14

“To create a report” on page 15

To start the DAR Calculator program

To start the DAR Calculator program, do one of the following:

- Click the DAR Calculator icon () on the desktop.
- Click **Start > All Programs > Agilent > MassHunter Workstation > DAR Calculator**.

To create CSV files in BioConfirm

The first step in using the MassHunter DAR Calculator software is to create CSV files in the MassHunter BioConfirm software containing the deconvoluted mass peaks.

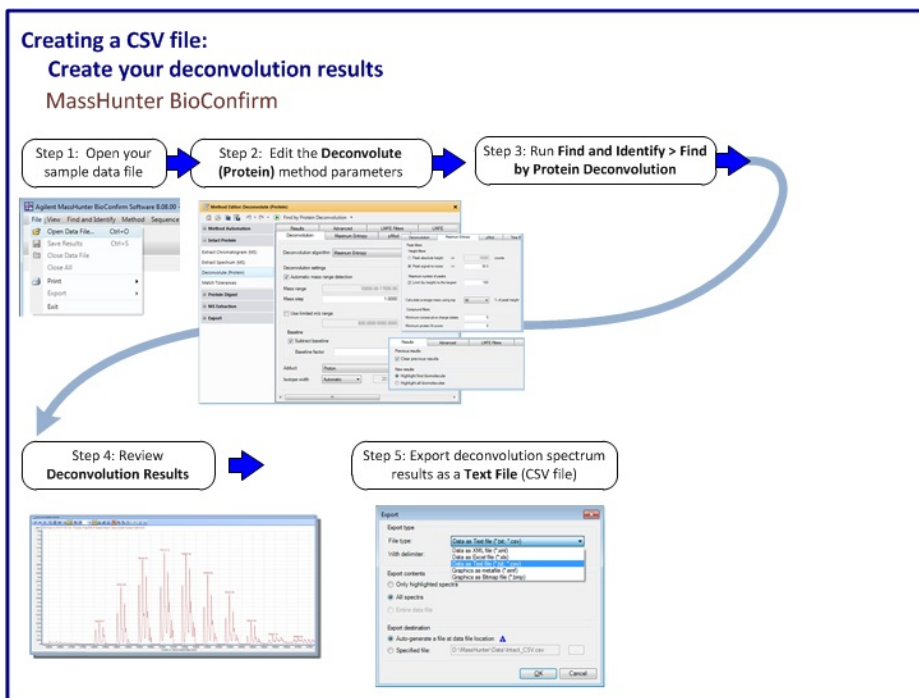


Figure 9 Creating a CSV file

- 1 Open your LC/MS sample data file.
- 2 Edit the Deconvolute (MS): Protein method parameters.
- 3 Run Deconvolute (Protein) from the Method Editor.
- 4 Review the deconvolution results plot and adjust the method parameters.
- 5 Export the deconvolution results as a text file (CSV file) from the deconvolution plot.

See the online Help for detailed information about each step.

Basic Tasks

To process Intact CSV file

To process Intact CSV file

You follow these general steps to process an intact CSV file.

- 1 Open a deconvoluted ADC spectrum (CSV file).
- 2 Select the ADC Type as Lys-Linked (All). (any number of drug molecules per antibody) or Cys-Linked (even) (only even numbers of drug molecules per antibody).
- 3 Enter the mass for the unconjugated antibody (DAR 0) in the toolbar.
- 4 Enter the mass for the drug/linker (the drug molecule and linker complex).
- 5 Review the results and adjust the method, peak properties, and plot.
- 6 Create a DAR report (PDF or DOCX file).
- 7 Save your investigation (DAR file).

See the online Help for detailed information about each step.

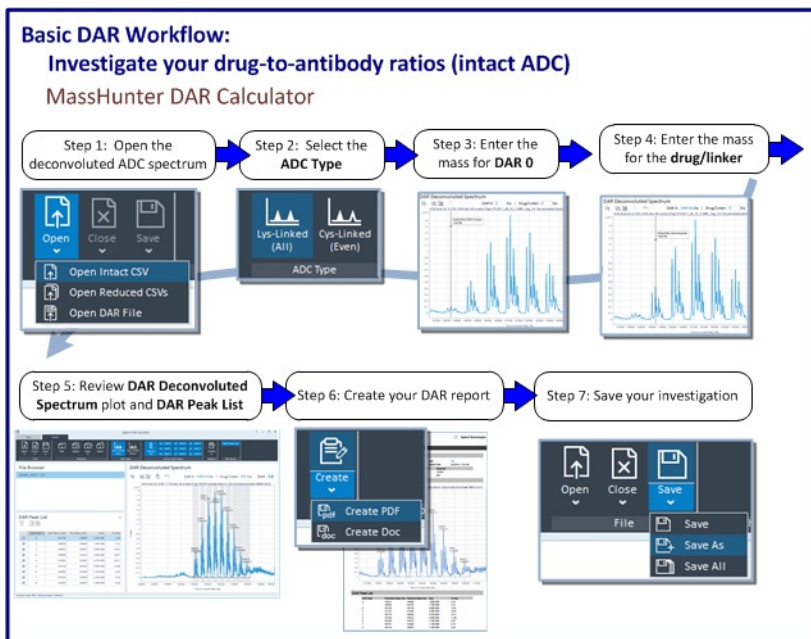


Figure 10 Process an Intact CSV file

To process Reduced CSV files

Follow these general steps to process reduced CSV files.

See the online Help for detailed information about each step.

- 1 Open the deconvoluted ADC spectra for the light and heavy chain immunoglobulins (CSV files).
- 2 Enter the mass for the unconjugated antibody (DAR 0) for the heavy chain.
- 3 Enter the mass for the unconjugated antibody (DAR 0) for the light chain.
- 4 Enter the mass for the drug/linker (the drug molecule and linker complex).
- 5 Review the results and adjust the method, peak properties, and plot.
- 6 Create a DAR report (PDF or DOCX file).
- 7 Save your investigation (DAR file).

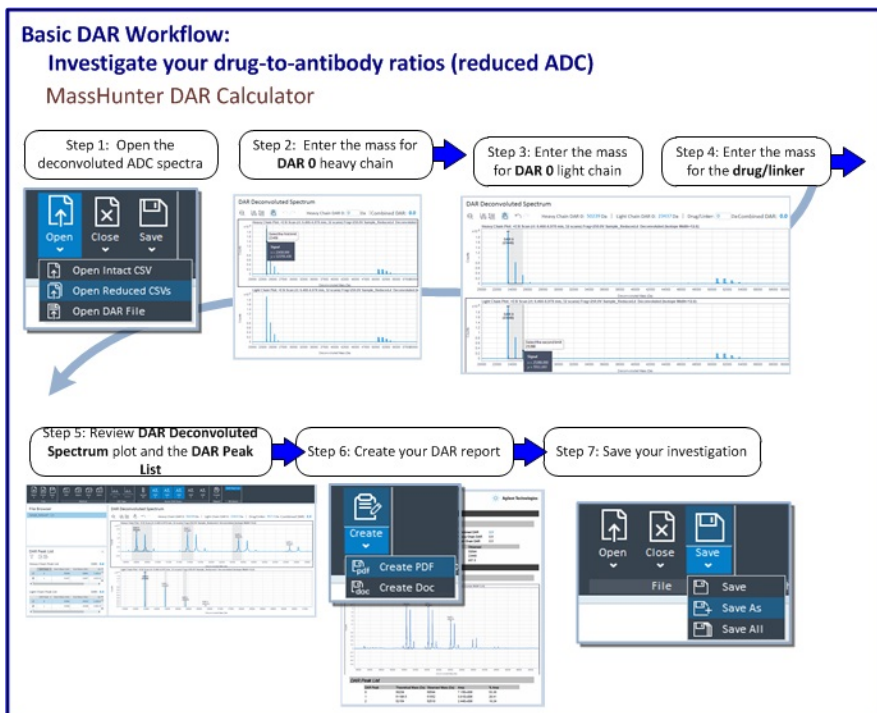


Figure 11 Process reduced CSV files

To edit results


To remove a peak

- Clear the peak icon in the toolbar.
- Clear the check box in the DAR Peak List pane.

To delete a peak

- Select the peak in the DAR Peak List table and press **Delete** on the keyboard.
- Select the peak in the DAR Peak List table. Right-click the peak and click **Delete Peak > Delete Peak**.
- Select the peak in the DAR Deconvoluted Spectrum pane. Right-click the peak, and click **Delete Peak > Delete Peak**.

To add a peak manually

- 1 Open one or two CSV files.
- 2 Process the data. See [“To process Intact CSV file”](#) on page 12 and [“To process Reduced CSV files”](#) on page 13.
- 3 If you have two reduced CSV files open, click either the Heavy Chain plot of the Light Chain plot. The peak will be added to only one peak list.
- 4 Click  on the DAR Deconvoluted Spectrum toolbar.
- 5 Click the location for the first limit.
- 6 Click the location for the second limit.

To change integration results

- 1 Open one or two CSV files.
- 2 Process the data. See [“To process Intact CSV file”](#) on page 12 and [“To process Reduced CSV files”](#) on page 13.
- 3 Zoom in around the peak that you want to modify in the DAR Deconvoluted Spectrum pane.
- 4 In the DAR Peak List table, click a value in the Start Mass (Da) column or in the End Mass (Da) column. The value in the cell is selected.
- 5 Click the location in the spectrum that you want to use as the Start Mass or the End Mass for the peak. When you click a different location, the software




automatically changes either the Start Mass or the End Mass for that peak and recalculates the **Area** and **% Area** (or **Height** and **% Height**).

To modify properties for the current file

- 1 Open one or two CSV files.
- 2 Process the data. See “To process Intact CSV file” on page 12 and “To process Reduced CSV files” on page 13.
- 3 Open the **Edit DAR Method** dialog box. Do one of the following:
 - Right-click the DAR Deconvoluted Spectrum pane and click **Edit Method**.
 - Right-click the DAR Peak List pane and click **Edit Method**.
 - Right-click the file name in the File Browser pane and click **Edit Method**.
- 4 Review the parameters and make any changes. You can change how **Peak area** is reported. You can also change how the Peak baseline is determined. You can also change the Maximum DAR peak number.
- 5 Click Apply. The changes are made to the current file only.

To create a report



- 1 Click  or  under  in the toolbar. The **Save As** dialog box opens.
- 2 Enter a File name. You can also switch to a different folder.
- 3 Click Save. The report is opened automatically.

To export results

Export DAR Peak List table

You can only export a DAR Peak List table if the DAR Peak List contains one or more peaks.

- 1 Select the table which you want to export.
- 2 Right-click the table in the DAR Peak List pane, and click **Export Table > CSV** or **Export Table to > XLS**.

Basic Tasks

To export results

3 Enter the **File name**. You can also change to a different folder.

4 Click **Save**.

Export DAR spectrum

You can export a DAR spectrum to one of these formats: **BMP**, **JPEG**, **PNG**, or **TIFF**.

1 Select the spectrum that you want to export.

2 Right-click the spectrum in the DAR Deconvoluted Spectrum pane, and click one of the following

- **Export Plot to > BMP**
- **Export Plot to > JPEG**
- **Export Plot to > PNG**
- **Export Plot to > TIFF**

3 Enter the **File name**. You can also change to a different folder.

4 Click **Save**.

Automated DAR Calculator Workflow

The topics in this section will help you get started using the Agilent MassHunter DAR Calculator Software with Agilent MassHunter BioConfirm software and Agilent MassHunter Acquisition for TOF/QTOF with Agilent MassHunter Walkup software.


- 1** Create a DAR Calculator method and save it.
- 2** Create a method with a Custom workflow in BioConfirm.
 - a** Select Custom as the workflow in the BioConfirm program.
 - b** Select Intact Protein Deconvolution with DAR Calculations as an action in the BioConfirm method.
 - c** Save the BioConfirm method to the same file as the DAR method.
- 3** Create an acquisition method in the Agilent MassHunter Data Acquisition program. Link or copy the combined BioConfirm and DAR method just created.
- 4** Create a Walkup Method that links to the Acquisition Method that you just created.
- 5** Create a Walkup Workflow to configure custom columns.

For more information, see the following topics:

- [“To create a DAR Calculator method”](#) on page 18
- [“To create a custom BioConfirm method”](#) on page 19
- [“To create a Data Acquisition method”](#) on page 22
- [“To create a Walkup Method”](#) on page 23
- [“To create a Walkup Workflow”](#) on page 25

To create a DAR Calculator method

The Intact DAR 0 mass and Drug/linker mass which you enter in the method are the default values that are used during automation. When you are automatically doing DAR calculations, you enter the appropriate values for your sample.

- 1 Click  in the Method group in the Ribbon.
- 2 Click the **Parameters** tab.
- 3 Click either **Lysine-Linked** or **Cystine-Linked**.
- 4 Enter the **Intact DAR 0** mass. This mass is used as the default Intact DAR 0 mass.
- 5 Enter the **Drug/linker mass**. This mass is used as the default Drug/linker mass.
- 6 Mark the selected peaks. These peaks are included in the calculation of DAR.

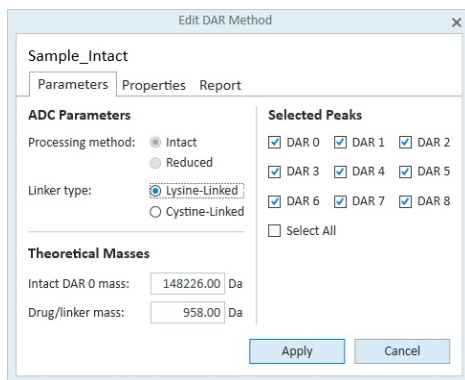



Figure 12 Edit DAR Method dialog box

- 7 Click the **Properties** tab. Review the **Integration Properties** and the **Max DAR Peak Number**.
- 8 Click the **Report** tab. Review the parameters.

- 9 Save the method. Click  in the Method group in the ribbon and select either **Save** or **Save as**.

To create a custom BioConfirm method

Use this procedure to create a BioConfirm method which also does DAR calculations. If you enter a **Sequence**, then the Match Sequences algorithm is also run.

- 1 Start the BioConfirm program. Click on the desktop or click **Start > All Programs > Agilent > MassHunter Workstation > BioConfirm B.08.00**. You need to use version B.08.00 or later of the BioConfirm program.
- 2 Open the default.m method. Click **Method > Open**. Select the *default.m* method in the BioConfirm\B.08.00 folder.
- 3 If the Method Editor window is not visible, click **View > Method Editor**.
- 4 Click the **Method Automation > Workflow and Sequences** section in the left pane.
- 5 Select **Custom** for the **Workflow**.
- 6 Select either **reduced** or **non-reduced** for the **Condition**.
- 7 (optional) Click the  button next to the **Sequence/Masses** parameter to select a sequence. See the online Help for BioConfirm for more information on adding a sequence using the Sequence Manager.

If you do not enter a sequence, then the **Match Sequences** algorithm is not run.

For the example data file (Sample_Reduced.d), here are the sequences:

Light Chain:

```
DIQMTQSPSSLSASVGDRTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASF  
LYSGVPSRFSGSRSRGTDFLTISLQPEDFATYYCQGHYTTPTFGQGTKVEIK  
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS  
QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG  
EC
```

Heavy Chain:

```
EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHVWRQA PGKGLEWVAR  
IYPTNGYTRY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCSRWG  
GDGFYAMDYW GQGLTVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK  
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
```

Automated DAR Calculator Workflow

To create a custom BioConfirm method

YICNVNHKPS NTKVDKKVPEP KSCDKTHTC PPCAPELLG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSLT CLVKGFPYPSD IAVEWESNGQ PENNYKTPP
VLDSGDSFFL YSKLTVDKSR WQQGNVFCSC VMHEALHNHY TQKSLSLSPG

- 8 (optional) Click the button next to the **Mods and Profiles** parameter to select modifications and profiles.
- 9 (optional) Mark the check box next to the **Enzymes** that you used.
- 10 In the **Available actions** list, select **Intact Protein Deconvolution with DAR Calculation**.

This one action does the following

- Runs the Intact Protein Deconvolution algorithm.
- Runs the Match Sequences algorithm (if a sequence is provided).
- Runs the DAR Calculations algorithm.
- Generates a report.

- 11 Click the to move this action to the **Actions to be run** list.

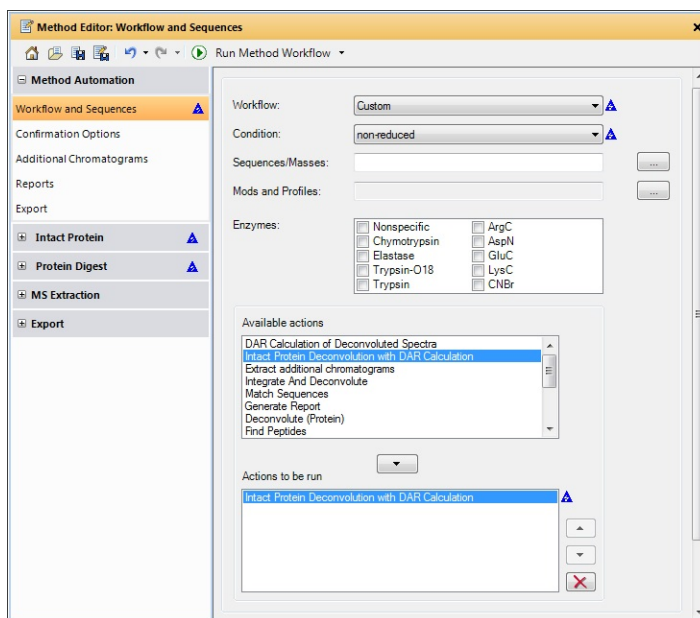


Figure 13 Method Editor window in the MassHunter BioConfirm program

12 Review the parameters in the Intact Protein section.

- Extract Chromatogram (MS)
- Extract Spectrum (MS)
- Deconvolute (Protein)
- Match Tolerances

13 Save the BioConfirm method to the same method name as the DAR method. BioConfirm does not overwrite the DAR method. Instead, BioConfirm method parameters are added to the DAR method producing a combined BioConfirm and DAR method.

To create a Data Acquisition method

Use this procedure to create a Data Acquisition method that links to the BioConfirm method.

- 1 Open the Agilent MassHunter Workstation Data Acquisition program. You can either double-click the Data Acquisition icon on the desktop or in the Agilent MassHunter Workstation group folder. You can also click **Start > All Programs > Agilent > MassHunter Workstation > Data Acquisition**.
- 2 Enter values for all of the LC modules configured for the instrument.
- 3 Enter the MS parameters in the TOF or Q-TOF tab. See the Data Acquisition Quick Start Guide for more information.
- 4 Click the **DA** tab.
- 5 Click the **BioConfirm** tab.
- 6 Mark the **BioConfirm Automation** check box.
- 7 Click the **Copy** or **Link** button.
- 8 Select the BioConfirm method that you created which also contains the DAR method. See [“To create a custom BioConfirm method”](#) on page 19.
- 9 Save the Data Acquisition method. You click either **Method > Save** or **Method > Save As**.

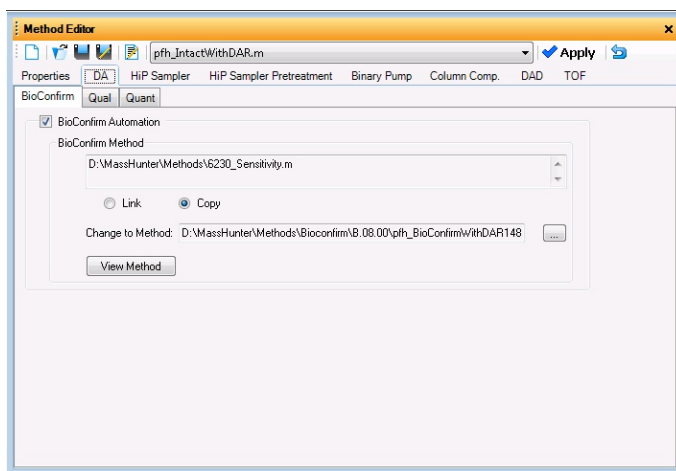





Figure 14 DA > BioConfirm tab in the Method Editor window in Data Acquisition

To create a Walkup Method

Use this procedure to create a Walkup method that uses the Data Acquisition method that you created. You must be a system administrator to create a Walkup Method.

See “[To create a Data Acquisition method](#)” on page 22.

- 1 Start the MassHunter Walkup program. From the Data Acquisition program, click **Walkup > Start Software**. You need to enter a valid name and password.
- 2 In the Walkup software, click Administration () in the Ribbon. You need to log in as an administrator.
- 3 Click **Configuration** in the left pane.
- 4 Click **Walkup Methods** () in the Configuration section in the left pane.
- 5 Click **<Add New Walkup Method>** in the right pane.
- 6 Enter information in the **<Add New Walkup Method>** section. Enter the **Walkup Method Name** and the **Estimated Runtime**.
- 7 In the Data System Method Details section, click  to select the folder where the method is saved. Click **OK**.
- 8 Select the Data Acquisition method you updated in “[To create a Data Acquisition method](#)” on page 22.
- 9 Enter the Injection Volume **Minimum**, **Nominal**, and **Maximum**.

Automated DAR Calculator Workflow

To create a Walkup Method

<Add New Walkup Method>

Walkup Method Name:

Walkup Method Description:

Walkup Method Priority: Estimated Runtime: min

Make available for sample submission

Workflow Scope

All Workflows

Specific Workflows:

Instrument Scope

All Instruments

Specific Instruments:

Data System Method Details

Data System Method:

Injection Volume

Minimum:	<input type="text" value="0.1"/>	µL
Nominal:	<input type="text" value="0.3"/>	µL
Maximum:	<input type="text" value="1.0"/>	µL

DA Operations

Equilibration Walkup Method Details

Equilibration Setting

Time to equilibrate after change: min

Equilibration Walkup Method:

Pre-Wakeup Walkup Method:

Post-Run Walkup Method:

Equilibration Group:

Figure 15 Walkup Methods section in the Walkup Administration program

10 Click **Save**.



11 Click **Verify** in the **Verify & Save** group in the Ribbon.

12 Fix any errors.

13 Click **Save** in the **Verify & Save** group in the Ribbon.

To create a Walkup Workflow

Use this procedure to create a Walkup Workflow so that a user can submit a DAR 0 value and a Drug/Linker value at the time of sample submission.

- 1 Start the MassHunter Walkup program. From the Data Acquisition program, click **Walkup > Start Software**. You need to enter a valid name and password.
- 2 In the Walkup software, click Administration () in the Ribbon. You need to log in as an administrator.
- 3 Click **Configuration** in the left pane.
- 4 Click **Workflows** () in the Configuration section in the left pane.
- 5 Click **<Add New Workflow>** in the right pane.
- 6 Enter a **Workflow Name**.
- 7 In the Workflow Columns section, enter DAR 0 Mass for the **Column Display Name**.
- 8 Select **Theoretical DAR 0 Mass** as the **Column Type**.
- 9 Enter Drug/Linker Mass for the **Column Display Name**.
- 10 Select **Theoretical Drug/Linker Mass** for the **Column Type**.

Automated DAR Calculator Workflow

To create a Walkup Workflow

Add New Workflow

Workflow Name: DAR Method

Workflow Description:

Instrument Scope

All Instruments

Specific Instruments: [Dropdown]

Workflow Columns

	Column Display Name	Column Type	Mandatory	Custom Value
1	DAR 0 Mass	Theoretical DAR 0 Mass	<input type="checkbox"/>	
2	Drug/Linker Mass	Theoretical Drug/Linker Mass	<input type="checkbox"/>	
*	Click here to add new column in Workflow.			

Move Up

Move Down

Custom Sample Import

Allow Custom Sample Import

Import Map: [Dropdown]

Sample Import Script: [Text Field] [Browse]

Save Cancel

Figure 16 Walkup Workflows section in the Walkup Administration program

11 Click **Save**.

12 Click **Verify** in the **Verify & Save** group in the Ribbon.

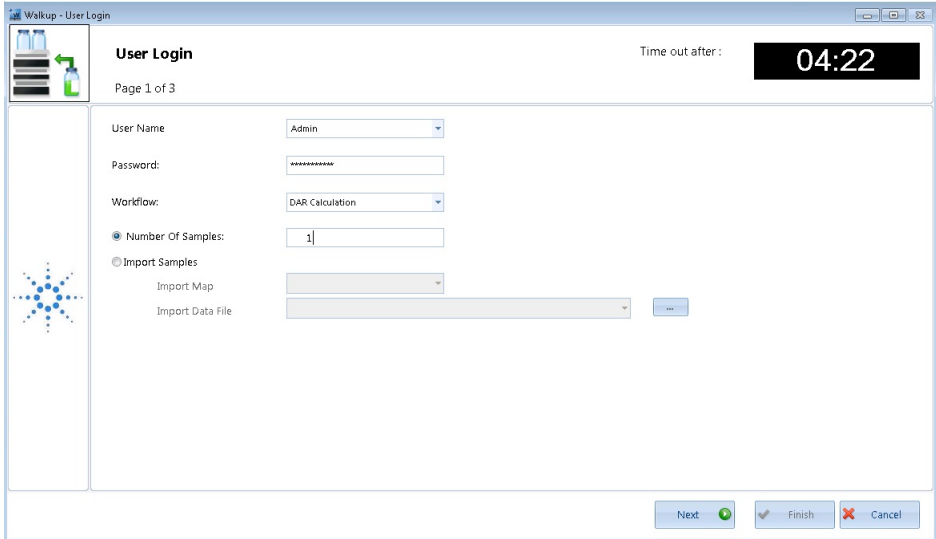
13 Fix any errors.

14 Click **Save** in the **Verify & Save** group in the Ribbon.

To submit a sample in Walkup that calculates DAR

Use this procedure to submit a sample that will automatically calculate DAR. You can enter the **DAR 0 Mass** and the **Drug/Linker Mass** if you want to use different values than saved in the method.

- 1 Start the MassHunter Walkup program. From the Data Acquisition program, click **Walkup > Start Software**. You need to enter a valid name and password.
- 2 In the Walkup software, click **Submit Samples**.
- 3 Select your **User Name** and enter the **Password**.
- 4 Select the DAR workflow that you created for the **Workflow**.
- 5 Enter the **Number of Samples**.



The screenshot shows the 'User Login' dialog box in the Walkup software. The window title is 'Walkup - User Login'. The page is 'Page 1 of 3'. A 'Time out after:' indicator shows '04:22'. The form contains the following fields and options:

- User Name: Admin (dropdown menu)
- Password: [masked with asterisks]
- Workflow: DAR Calculation (dropdown menu)
- Number Of Samples: 1 (text input field)
- Import Samples: (radio button, selected)
- Import Map: [dropdown menu]
- Import Data File: [text input field]

At the bottom right, there are three buttons: 'Next' (with a green arrow), 'Finish' (with a checkmark), and 'Cancel' (with a red X).

Figure 17 User Login page of the Sample Submission dialog box

- 6 Click **Next**. The **DAR 0 Mass** column and the **Drug/Linker Mass** column are automatically displayed in the table because you selected the DAR workflow.
- 7 Enter the **Sample Name**.
- 8 Enter the **DAR 0 Mass**.

Automated DAR Calculator Workflow

To submit a sample in Walkup that calculates DAR

- 9 Enter the **Drug/Linker Mass**.
- 10 Select the **Walkup Method** that you created.
- 11 Enter the **Injection Volume**.

	Sample Name	DAR O Mass	Drug/Linker Mass	Walkup Method	Injection Volume
1 *	DAR Test	148226	991	Walkup DAR Met...	0.30

Figure 18 Table on the Sample List page

- 12 Click **Next**.
- 13 Place your vial in the position indicated.
- 14 Click **Finish**.

To create and run a Data Acquisition worklist

Use this procedure to create a Data Acquisition worklist that adds columns for the DA Workflow, the drug/linker, and the DAR 0 mass (or masses).

- 1 Open the Agilent MassHunter Workstation Data Acquisition program. You can either double-click the Data Acquisition icon on the desktop or in the Agilent MassHunter Workstation group folder. You can also click **Start > All Programs > Agilent > MassHunter Workstation > Data Acquisition**.
- 2 Click the Worklist tab to show the Worklist window. If the Worklist window is not visible, click **View > Worklist**.
- 3 Right-click the upper left corner of the worklist and click **Add Column(s)**. You can also click **Worklist > Add Column(s)**.
- 4 Select **DAR 0 Mass** for the **Column Type**.
- 5 Enter the **Column name**. If you want to enter a light DAR 0 and a heavy DAR 0, then you still only add one DAR 0 Mass column. The user enters two values into this column separated by a comma.
- 6 Click **OK**.

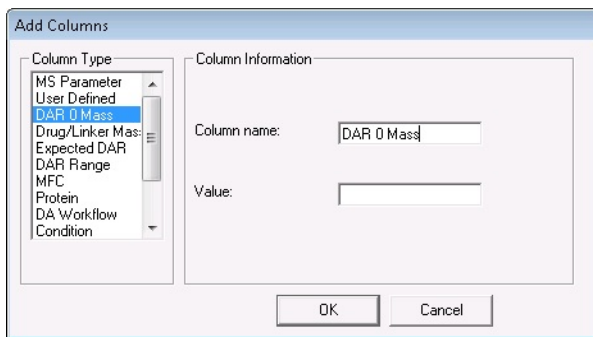


Figure 19 Add Columns dialog box

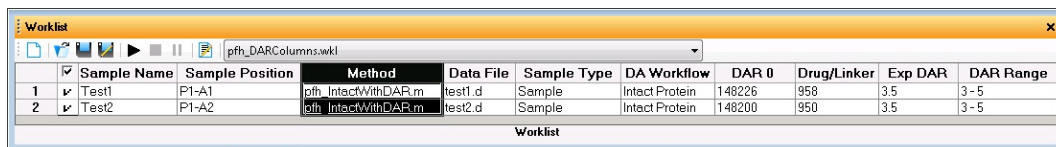
- 7 Click **Worklist > Add Column(s)**.
- 8 Select **Drug/Linker Mass** for the **Column Type**.
- 9 Enter the **Column name**.
- 10 Click **OK**.
- 11 (optional) Add a column for **Expected DAR** or **DAR Range**. These values will be included in the DAR report. Do not add the **DA Workflow** column.

Automated DAR Calculator Workflow

To create and run a Data Acquisition worklist


This column is used to override the BioConfirm Workflow specified in the method; however, to do DAR calculations, the **DA Workflow** must be Custom.

- 12 Add one or more samples to the worklist. Select the method created in “[To create a Data Acquisition method](#)” on page 22. Enter the **DAR 0 Mass** and the **Drug/Linker Mass**. If you want to enter DAR 0 for a light chain and for a heavy chain, enter both values in the DAR 0 Mass column and separate them with a comma. For example, you can enter 12000, 94000.
- 13 Click **Worklist > Worklist Run Parameters**. The **Worklist Run Parameters** dialog box opens.
- 14 Select **Both Acquisition and DA** or **DA Only** for **Part of method to run**. You select **DA Only** if you have already acquired the data files.
- 15 Review the other parameters.
- 16 Click **OK**.
- 17 Save the worklist. Click **Worklist > Save** or **Worklist > Save As**.



	Sample Name	Sample Position	Method	Data File	Sample Type	DA Workflow	DAR 0	Drug/Linker	Exp DAR	DAR Range
1	Test1	P1-A1	pth_IntactWithDAR.m	test1.d	Sample	Intact Protein	140226	958	3.5	3-5
2	Test2	P1-A2	pth_IntactWithDAR.m	test2.d	Sample	Intact Protein	140200	950	3.5	3-5

Figure 20 Worklist with DAR columns

- 18 Run the worklist. Click **Worklist > Run**. You can also click  on the main toolbar to run the worklist.

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In This Guide

This guide has instructions for installing and using the Agilent MassHunter DAR Calculator Software.

This guide is valid for the B.01.01 revision or higher of the G4994AA Agilent MassHunter DAR Calculator Software, until superseded.

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Printed in USA

Revision A, June 2016



G4994-90000



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