

qPCR workflows

Diomni Software for diagnostic qPCR assay developers and clinical laboratories

Abstract

The Applied Biosystems™ Diomni™ Software (v2.0.0) is part of a digitally integrated PCR-to-result workflow for diagnostic laboratories and assay developers who perform *in vitro* diagnostic (IVD) assays and laboratory-developed tests (LDTs). This web-based software package seamlessly integrates assay setup and data analysis with the use of assay definition files, which are assay-specific files that contain all quantitative PCR (qPCR) run, data analysis, and regulatory use parameters. Diomni Software includes a security, auditing, and e-signature (SAE) module that can help diagnostic laboratories maintain a secure environment to comply with regulatory requirements. Use Diomni Software with the Applied Biosystems™ QuantStudio™ 7 Pro Dx Real-Time PCR System to centralize your workflow, use and manage assay definition files, set up runs, analyze results, and prepare customizable reports.

Introduction

In the age of precision medicine, molecular assays will continue to grow in number and importance. Assay developers face complex challenges as they work to find solutions for emerging and unmet needs, and clinical laboratories are often required to quickly implement new IVD tests with short turnaround times. PCR-based molecular diagnostic (MDx) assays have become indispensable in the diagnostic landscape and are widely used in clinical laboratories. The simplicity, high degree of accuracy, and analytical sensitivity of PCR assays make them ideal for diagnostic applications in many settings, including oncology and infectious disease laboratories. Robust PCR technology and chemistry also enable the detection of multiple targets in the same reaction.

Diomni Software is a simple, yet powerful centralized platform for connectivity and advanced data analysis that can help streamline the implementation of new IVD assays and LDTs. Clinical laboratories can utilize the plug-and-play design of the software to expand their clinical testing menu without extensive system validation (Table 1). At the core of Diomni Software are assay definition files (ADFs). Every ADF is specific for one assay panel and provides a digital representation of all qPCR run, analysis, and regulatory use parameters. An ADF defines the following:

- Assay panel name, version, and description
- Instrument, block type, and run parameters like the thermal cycling profile and reaction volume
- Positive and negative controls for the assay
- Assay targets, including reporter and quencher filters for all targets and controls

- Test codes for reporting results
- Primary analysis settings, including C_q analysis parameters
- Secondary analysis parameters like call settings for targets, samples, and controls
- Research Use Only (RUO), Investigational Use Only (IUO), or IVD regulatory use status

The use of ADFs enables a seamless transition from assay development and performance evaluation to clinical testing. As an integral part of assay development, Diomni Software enables scientists to create, publish, and manage ADFs for different regulatory use cases (Table 2). RUO ADFs are used for assay development and can be edited, and assay developers can use RUO ADFs to generate or “publish” IUO ADFs to evaluate performance. An RUO or IUO ADF can also be published as an IVD ADF, although it is important to note that IVD ADF-based assays must comply with regulatory requirements for IVD use.

Table 1. Features of Diomni Software.

Centralized qPCR workflow	<ul style="list-style-type: none"> • Intuitive user interface • One software package that supports all clinical qPCR workflow needs • Web-based design that centralizes data tracking • Connectivity with all QuantStudio 7 Pro Dx Real-Time PCR instruments • Create, publish, and manage ADFs • Plug-in design to expand your testing menu • Customizable export formats for flexible LIMS or LIS integration
Simplified qPCR setup	<ul style="list-style-type: none"> • Simplify qPCR setup with RUO, IUO, or IVD ADFs • Send qPCR setup information to networked or stand-alone QuantStudio 7 Pro Dx system(s) • Receive qPCR run data automatically from networked QuantStudio 7 Pro Dx system(s) • Track reagent lots • Track qPCR system calibration
Automated data analysis and customizable reporting	<ul style="list-style-type: none"> • Automated data analysis with defined ADF parameters • Review results with enhanced data visualization • Adjustable analysis parameters for privileged users in the RUO workspace • Highly customizable report with header, logo, and data content
Safe and secure	<ul style="list-style-type: none"> • Customizable SAE module

Table 2. Overview of ADFs for different regulatory use cases.

RUO ADFs	RUO ADFs are created during assay development and can be edited. RUO ADFs are for research use only and cannot be used for clinical diagnosis or patient management. Developers can generate (publish) IUO or IVD ADFs from RUO ADFs.
IUO ADFs	Published IUO ADFs are intended for evaluation or validation purposes only and cannot be edited. IUO ADFs cannot be used for clinical diagnosis or patient management and are intended for medical device manufacturers or assay developers who perform investigational studies. Developers can generate IVD ADFs from approved IUO ADFs.
IVD ADFs	A published IVD ADF can be distributed as part of a test or assay kit by a manufacturer or assay developer after FDA clearance or approval is received for the test or the assay. IVD ADFs cannot be edited.

Using Diomni Software to manage your qPCR assays and data helps reduce the risk of human error by standardizing your workflow (Figure 1). The first step is to install an assay-specific ADF in the “Assay Definition” selection menu. The next step is to create a new run by selecting the assay-specific ADF with your predefined run parameters and analysis settings. Configure the sample and control positions in the plate, add your sample information, enter the reagents used for the run (optional), and print the plate layout for use in the laboratory (optional). Sample information can also be imported from your laboratory information management system (LIMS) or laboratory information system (LIS). Prepare the qPCR reactions and start the run in your laboratory space. Once the run is complete, Diomni Software will automatically analyze the run data based on your ADF settings. You can then review your results and export the run data or generate a customizable clinical report.

The innovative Diomni Software puts you in command, and it is an effective tool for overcoming assay design and sample testing challenges. You can manage your ADFs and expand your testing options with this web-based software package and easily oversee laboratory operations. Diomni Software enables you to connect your qPCR instruments, track your qPCR workflows, and perform automated data analysis.

The SAE Administrator Console

The SAE Administrator Console is a client-server configuration that is at the core of Diomni Software connectivity. The QuantStudio 7 Pro Dx Real-Time PCR System connects to Diomni Software through the SAE Administrator Console, and you can configure the SAE settings for your specific application there.

The system security function of the SAE Administrator Console controls user access, and it allows administrators to create, disable, and activate SAE accounts. Administrators can also configure SAE permissions for individual accounts. The auditing function tracks the activities of users as well as changes in the SAE module settings. Audit mode settings can be adjusted as required, and audit summaries are accessible as reports. The e-signature function allows e-signature requirements to be added for specific tasks.

Diomni Software can be installed on the same system as the SAE Administrator Console or on any system in the same network. It should be noted that Diomni Software must be connected to the SAE Administrator Console to be operational, and Diomni Software requires the user to log in with an active SAE account. The SAE Administrator Console also controls the SAE and account settings for use of connected QuantStudio 7 Pro Dx Real-Time PCR instruments.

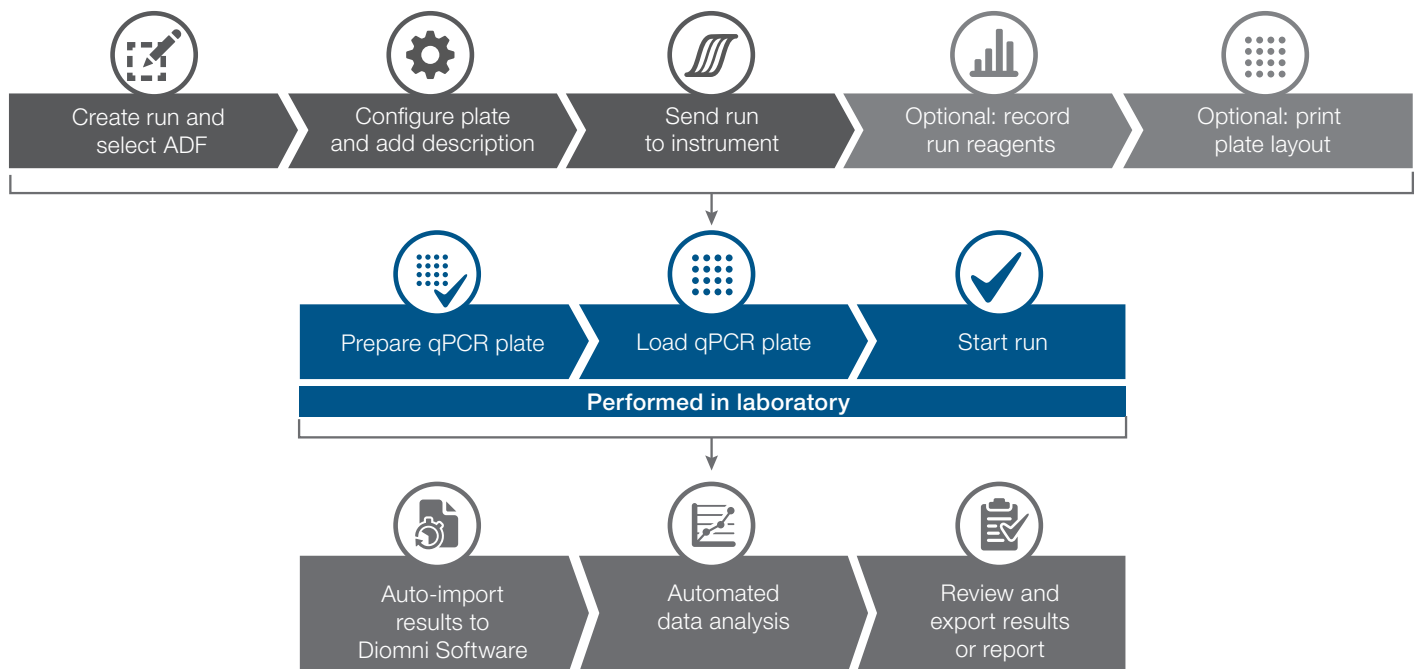


Figure 1. qPCR assay workflow with Diomni Software.

Assay definition files (ADFs)

ADFs are at the core of Diomni Software, and we will provide a detailed overview of their properties and functions in this section. ADFs contain nine different sections that can be defined and reviewed (Table 3). They contain all parameters for running qPCR assays, including criteria for presence and absence calls for targets and controls, analyzing results, and reporting them.

The **Summary** section contains information about the ADF, including the file name, version, description, and the supported qPCR instrument and block type.

The **Run Method** section defines the reaction volume and PCR conditions, such as the heated cover temperature and cycling protocol. Steps can be easily added or removed in the intuitive user interface using the plus and minus (+/-) symbols. Users can add or remove one step at a time using the +/- symbols at the bottom of the screen. The +/- symbols at the top of the screen allow users to add or remove predefined stages, such as “PCR” and “Hold”, that may contain multiple steps. All values, including temperature, time, and cycle number, can be adjusted by simply clicking the associated boxes. The “Advanced settings” symbol in each step opens a pop-up window where additional settings can be defined. Users can also import .eds or .edt files to create ADFs.

Positive and negative controls are defined in the **Controls** section. A description for each control can be added, and a rule to invalidate sample calls for a failed control can be activated. If the rule is activated and a control fails, the description entered under “Plate results” will appear in the report instead of the sample result.

Any passive reporter dye used to normalize the fluorescence signal and all targets are defined in the **Targets** section.

Information about the reporter and quencher for each target and any target that serves as an internal control can be marked. Results from multiple wells can be grouped using the “Target set groups” function for multiplex assays when the number of targets exceeds the number of available fluorescence channels. Each group represents a single-well assay assignment.

The **Test Codes** section defines the results reported for a test. If data is collected for more targets than a sample needs to be tested for, test codes can be used to define reporting for the sample. For example, an ADF with four different targets can have four different test codes. Each test code will drive a result for one of the four targets. Alternatively, a single test code can be created to generate a combined result for the four targets. A specific test code can be linked to a sample to define sample-specific reporting of results.

Baseline and threshold settings are added in the **Primary Analysis** section. The C_q cutoff and ΔRn threshold for each target are defined in the **Target Call Settings** section. The parameters defined in this section allow the user to call the presence or absence of targets.

Define the targets that must be present or absent in the positive and negative controls in the **Control Settings** section. Use the **Sample Call Settings** section to define which results will be reported for samples based on the presence or absence of assay targets. Sample call settings must be set up for all test codes in the ADF. Alternatively, the automatic rule generation feature can be used to automatically create call rules based on the defined targets. The user will need to review and adjust call results as needed.

Table 3. Sections of an ADF.

Section	Description
Summary	The Summary section includes the identifying information for the ADF, including the file name, version, description, and supported qPCR instrument and block type.
Run Method	The thermal protocol and optical filter settings for a real-time PCR run are defined in the Run Method section and include reaction volume, heated cover temperature, and cycling conditions.
Controls	The assay-specific control samples and validation settings are defined in the Controls section.
Targets	Targets and associated reporters, quenchers, and passive reference dye are defined in the Targets section.
Test Codes	Test codes can be used to define the assay targets that will be reported.
Primary Analysis	The algorithm settings for real-time PCR data analysis with target-specific baselines and thresholds are defined in the Primary Analysis section.
Target Call Settings	The analysis settings used to determine the presence or absence of a target template in a sample are defined in the Target Call Settings section. These include target-specific C_q cutoffs and ΔRn thresholds.
Control Settings	These settings define the interpretive rules used to report results for control samples.
Sample Call Settings	These settings define the rules used to interpret sample results.

Diomni Software workspaces

Diomni Software has two main workspaces, the *Development* workspace and the *Sample to Report Workflows* workspace (Figure 2). The *Development* workspace supports ADF creation and assay definition management, while the *Sample to Report Workflows* workspace allows the user to set up a qPCR run for RUO, IUO, or IVD use.

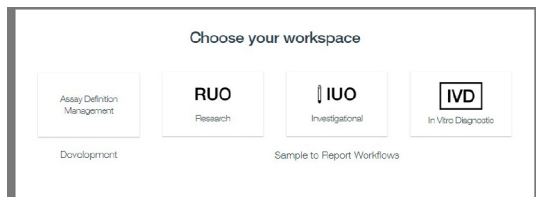


Figure 2. Diomni Software workspaces.

Development workspace

The assay definition management function allows the creation and management of RUO, IUO, and IVD ADFs in the *Development* workspace. ADF management options include create, update, delete, import, export, and publish. Depending on their SAE permissions, users can search for ADFs, review ADF properties, and import, export, delete, or create ADFs. Existing ADFs can be published to a regulatory use category, but any new ADF created with Diomni Software is restricted to RUO. To create a new ADF, select “Add assay definition” in the Research section of the *Development* workspace. An existing ADF can be cloned to create an identical copy, which will be saved as a new RUO ADF that can serve as an editable template.

All parameters in an RUO ADF can be edited. An RUO ADF can be published as either an IUO or an IVD ADF using the publish function, and IUO ADFs can be published as IVD ADFs. IUO and IVD ADF parameters are locked. They can only be reviewed, not changed, and IUO and IVD ADFs must comply with applicable regulatory requirements. An ADF in the *Assay Definition Management* workspace must be exported before it can be installed on the QuantStudio 7 Pro Dx Real-Time PCR System or in the *Sample to Report Workflows* workspace.

Sample to Report Workflows workspace

The *Sample to Report Workflows* workspace is used to set up a run and review results. To set up a run using Diomni Software, an ADF must be installed in the *Sample to Report Workflows* workspace. The same ADF needs to be installed on the QuantStudio 7 Pro Dx Real-Time PCR System, and the ADF must have the same regulatory use status as the selected workflow. For example, RUO ADFs can only be utilized in the RUO *Sample to Report Workflows* workspace.

Once the regulatory use status is selected, all runs installed in that environment will be displayed. Each run will have a unique ID and name. The table display can be customized under preferences to show information about the instrument, the status of the run, the run time, and any modifications. Run status indicates what stage the run is in, and progress can be tracked remotely. The run status differentiates between pre-run and post-run conditions (Table 4).

Table 4. Run status.

Status	Description	
Pre-run	Plate laid out	The run has been created, and all information can still be edited.
	Queued up	The run file has been sent to the QuantStudio 7 Pro Dx Real-Time PCR System. The run is either queued up or running on the PCR system.
Post-run	qPCR completed	The run has been completed.
	qPCR aborted	The run has been aborted.
	qPCR error	The run had an error.
	Closed	The run has been completed, reviewed, and closed.

The *Sample to Report Workflows* workspace also contains information about available instruments and installed ADFs (Figure 3). All ADFs installed in the *Sample to Report Workflows* workspace are listed in the *Assay Definitions* tab. ADFs cannot be edited in this workspace and are categorized according to regulatory use case. ADFs can be installed, reviewed, or uninstalled, and automatic reporting or exporting of results can be enabled or disabled. The *Instruments* tab lists all qPCR instruments that are registered for use with Diomni Software. The block format and status of each instrument are also shown. Additional information about each instrument can be reviewed, including the instrument and block serial numbers, the instrument software version, and a calibration summary. It should be noted that only the RUO *Sample to Report Workflows* workspace allows assays to be run when the calibration has expired.



Figure 3. User interface for the RUO *Sample to Report Workflows* workspace.

Pre-run setup and run management

To set up a new run, simply use the “Add run” function (Figure 3) and complete the three steps shown in Table 5. Runs can be accessed and opened in the *Runs* tab, and each step can be edited for any existing run with “Plate laid out” status.

Table 5. Run setup in three steps.

Step	Description
1. Select assay	Select the appropriate ADF for the test.
2. Configure plate	Assign the control and sample positions in the plate.
3. Finalize run	Update the run name (optional) and select the correct qPCR system. Save and send to the run queue.

The first step is to select the ADF that will be used for the run. The second step is to configure the positions of the controls and samples in the plate. Diomni Software will assign the well positions for the controls defined in the ADF. The positions of the controls can be modified by deleting the predefined positions, selecting new wells, and adding names that identify them as positive or negative controls as defined in the ADF. Select the wells the samples will be added to, then add the sample names and types. ADFs that support target set groups allow the user to define the target group in a selected well for each sample. For example, if two target groups are defined in the ADF, each sample must be tested in two wells to cover target group 1 (qPCR reaction 1 in well 1) and target group 2 (qPCR reaction 2 in well 2). In the third step, select the correct qPCR system and send the file to the run queue.

A run can be saved at any time using the “Save” function in the “More actions” menu. Diomni Software will automatically generate a run name that reflects the ADF name with a time stamp, but the run name can be modified. The run will be in “Plate laid out” status. Information can be edited unless a run is saved using the “Save & send to run queue” function, which will change the run status to “Queued up”. Diomni Software can import plate setup .csv files and export plate layout .xlsx files, and a run file can be downloaded or imported via the “More actions” menu in the *Runs* tab (Figure 3). SAE-configurable e-signatures for run files accessed by authorized users document that the information has been reviewed and approved.

Post-run results and reporting

Once a run is complete, the status will change to “qPCR completed.” “qPCR aborted” or “qPCR error” status indicates a run is not complete. Selecting a run with “qPCR completed” status allows the user to review the results, edit the plate setup, export the run data, and generate a report. Note that performing runs in the *RUO Sample to Report Workflows* workspace allows users to edit ADFs and analyze results based on updated settings. The IUO and IVD regulatory use workspaces do not allow changes to ADF settings.

Four tabs are associated with completed run files (Table 6). A summary of the results will be displayed in the *Summary* tab. The top row summarizes the results for the run controls and shows the name of the ADF file used and the number of samples run on the plate. The sample count includes controls and unique samples. The row below provides an overview of sample positivity and negativity based on test code. The *Summary* tab also shows information about the run, including the run date, operator, the qPCR system used, and the system calibration status.

Table 6. Tabs in a post-run file.

Tab	Description
Summary	Provides a summary of the results, run events, and qPCR system.
Details	Provides graphical and tabular results at the end of the run. The RUO workspace allows data points to be analyzed based on the editable assay definition settings for the run. This feature is not available in the IUO and IVD workspaces.
Export	Select the file format and data points to export.
Report	Customize the report settings.

The amplification curves are shown in the *Details* tab, and results are shown in table format. The results include sample calls, well calls, and target calls. There is an option for editing the ADF settings used for the run in the “More action” menu in the RUO workspace, and results can be re-analyzed by applying the updated parameters. This option is role-dependent and can be configured in the SAE console. However, this option is not available in the IUO and IVD regulatory use workspaces. The “Create ADF” function gives assay developers the option of saving modified ADFs as new RUO ADFs in the *Development* workspace. Authorized users can edit plate setup in all workspaces. Test codes can be applied for each sample in the *Details* tab to determine which results will be reported.

A preview of the data will appear in the *Export* tab, and the data export settings can be customized. Select the relevant data points to export, then export the data in .csv, .txt, or .xlsx format and save to a local or network folder.

The *Report* tab allows users to select the information that will be included in the report, and a preview of the report will be displayed. Preferences for the logo, header, and paper size can be defined in the software settings (Figure 3). Diomni Software also enables e-signature in the SAE configuration for approval of completed runs.

A “qPCR completed” run file can be closed to prevent it from being changed, and only authorized users can reopen it. Diomni Software also allows the user to track the reagents used to set up a run. This option is available in the “More actions” menu, and reagents can be edited at any time until the run is in “Closed” status. An audit report can also be created and downloaded for any run file, regardless of its status.

Security features

The security, auditing, and e-signature (SAE) features of Diomni Software can help users comply with regulatory requirements and security guidelines for electronic records by preventing unauthorized access to instruments, and it can support multiple clients. Diomni Software also allows data exchange with your LIS or LIMS system, and the user interface is intuitive and easy to use. The SAE features of Diomni Software include:

- System security to control user access to the software. A default administrator account is provided, and additional user accounts and permissions can be defined.
- Auditing capability to track user activity and changes to the SAE module settings. The software automatically and silently audits some actions, and users can select other actions to audit and specify the audit mode.
- Electronic signature capability for users who are required to provide usernames and passwords when performing certain functions. The e-signature settings can be configured so that a user can print a report or start a run only if the associated data are signed. E-signature events can also be configured to require multiple signatures or require users with specific permissions to sign.

Summary

Diomni Software is a comprehensive solution for diagnostic assay developers and clinical laboratories. The centralized web-based software tracks the status of all PCR runs and enables users to monitor laboratory operations. Assay developers can create assay definition files (ADFs) for their assays to integrate their setup, run, and analysis settings to streamline and simplify complex molecular testing workflows. In a clinical diagnostic setting, ADFs make instrument setup and reporting easier, help prevent user error, and support PCR-to-answer workflows.

With Diomni Software, running a qPCR assay is as simple as creating a run, selecting the appropriate ADF, and configuring the plate positions. Once users run their qPCR assays in the laboratory, Diomni Software will analyze the results and create highly customizable reports. Diomni Software also has e-signature features to document review and acceptance of plate setup and results for quality assurance purposes. The audit trail tracks every step and user modification to help ensure full regulatory compliance.

Learn more at thermofisher.com/diomni

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