C190-E304B

SHIMADZU
Excellence in Science

Fully Automated Sample Preparation Module for Glycan Analysis

MUP-3100

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Fully Automated Sample Preparation Module for Glycan Analysis

Automatic Pretreatment of Modified Glycans from Antibodies in Culture Supernatants

Stable performance eliminates differences between analysts, providing peace of mind

The structure of glycans in antibody drugs may affect the stability, function, and structural integrity of biopharmaceuticals; as a result, glycan profiles are evaluated for quality control purposes.
Glycan structures vary considerably depending on the cultivation conditions, and non-uniformity cannot be avoided, so it is controlled even in the production process.

HPLC and LC/MS are used for the profiling and structural analysis of modified glycans from antibodies in the culture supernatants of antibody-producing cells. The pretreatment sequence, including the purification of antibodies from culture supernatants, glycan cut-out, and fluorescent labeling, is complicated and typically requires at least two days.

Shimadzu developed the MUP-3100 Fully Automated Sample Preparation Module for Glycan Analysis in conjunction with Sumitomo Bakelite Co., Ltd., for use with their Auto-EZGlyco™ mAb-N Kit.
The MUP-3100 eliminates manual sample preparation, increases overall analysis throughput, and improves reproducibility.

Product

Precisely Automated Protocols Eliminate Human Error

- Performs dispensing, the addition of reagents, heating, centrifugation, solid phase column extraction, and sample delivery, automating the sequence of glycan pretreatment*
- Eliminates human errors and variations by automation
- Prevents mistakes by checking the positioning of the reagents via image processing before beginning automated operations, supporting a stable workflow

* The reagents included in the kit are prepared in advance by the analyst.

Provides Labor Savings and Reduces Downtime

- Simply set the reagents prepared with samples and kits into the device to automatically perform pre-processing and complete it without manual intervention
- Stores the automatically generated preparation results report in the database
- Equipped with error log and movie saving function in case of trouble and to ensure traceability

Processes 24 Culture Supernatant Samples Reliably in 6 Hours

- Through the combination of MUP-3100 and the kit, complicated procedures can be automatically processed in 6 hours
- Accommodates *N*-linked glycans (*N*-glycan) in antibodies*, allowing simultaneous processing of up to 24 samples
- Possible to verify the profiling of glycosylation from antibodies in culture supernatant, towards achieving an efficient workflow for optimizing culture conditions

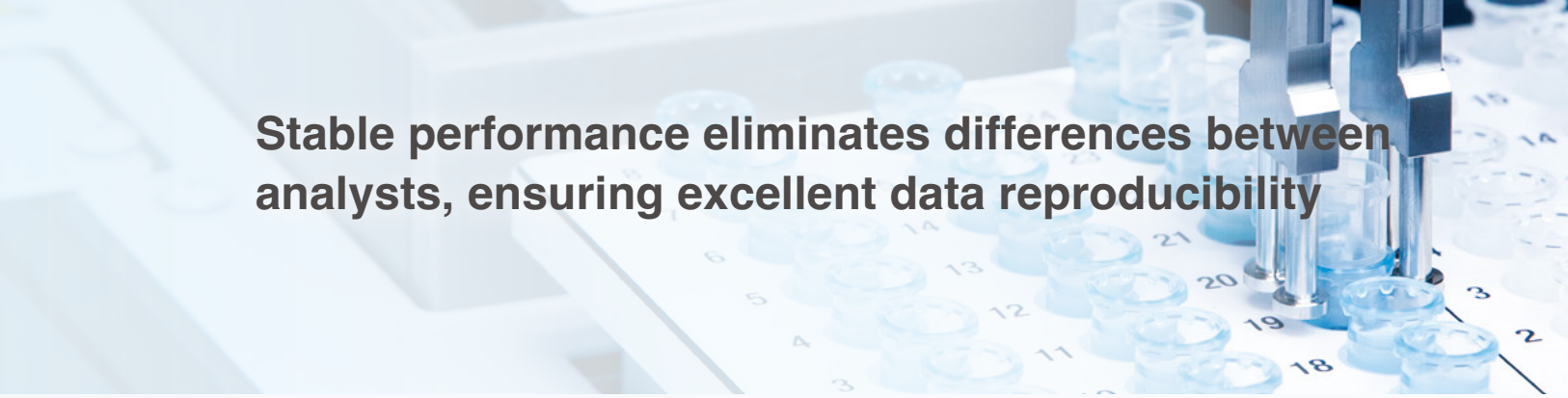
* Support for *O*-glycan is also available with the introduction of the *O*-glycan Upgrade Kit (p.7).

Auto-EZGlyco™ mAb-N Kit for SHIMADZU

(Sumitomo Bakelite Co., Ltd.)

This is capable of everything from purifying antibodies from unpurified samples (e.g. cell culture solution) and other partially purified samples, to glycan cut-out, fluorescent labeling of glycans and glycan purification. The kit also includes necessary reagents such as a glycan release enzyme and a glycan fluorescent labeling reagent.

Product

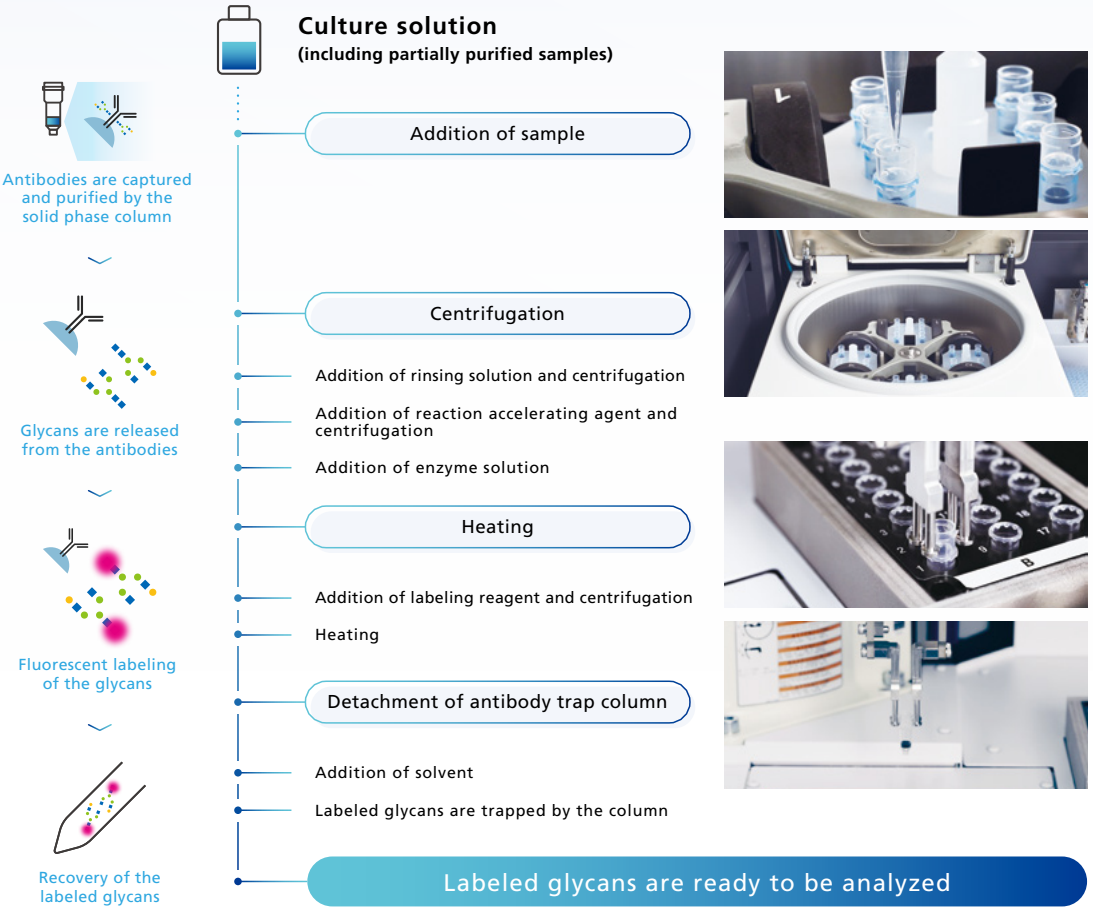


Stable performance eliminates differences between analysts, ensuring excellent data reproducibility

Precisely Automated Protocols Eliminate Human Error

Pretreatment is required for LC analysis of profiles for antibody-derived glycans, including purification of the antibodies, glycan cut-out, and fluorescent labeling. The MUP-3100 Fully Automated Sample Preparation Module for Glycan Analysis is equipped with functions to perform dispensing, the addition of reagents, heating, centrifugation, solid phase column extraction, and sample delivery, thereby automating the entire pretreatment sequence.
Pretreatment of antibody-derived glycans in culture supernatants involves repeated operations including dispensing, the addition of reagents, centrifugation, and heating. With solid phase column extraction using an antibody trap carrier, rinsing must be performed multiple times, which is labor intensive.
The MUP-3100 reliably implements pretreatment using the Auto-EZGlyco mAb-N Kit for SHIMADZU protocol. This eliminates human errors, such as forgetting to add the reagent, taking the wrong sample, or omitting a process.

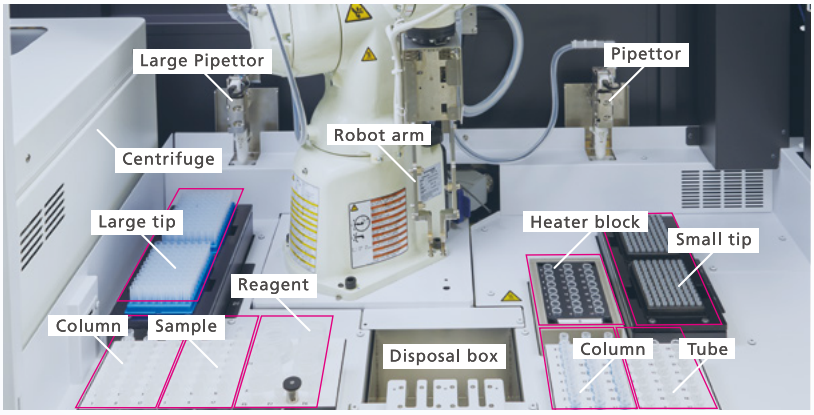
Automation of the Entire Process



Provides Labor Savings and Reduces Downtime

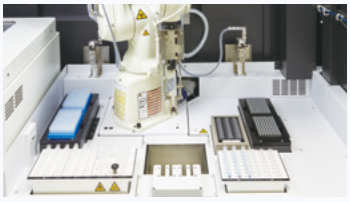
Easy sample and reagent loading operations

Simply place the prepared reagent and the culture supernatants or other samples into the prescribed place for the instrument and select pretreatment to implement the process automatically, quickly and accurately. The MUP-3100 allows for setting a maximum of 24 samples at one time and enables processing twice a day. The MUP-3100 not only lessens the burden on the analyst, but also enables pretreatment at night or on holidays, thereby accelerating the workflow.



Automatic Confirmation of Consumables Positioning Supports Stable Operations

The MUP-3100 is equipped with image processing technology that enables the automatic checking of samples and reagents, preventing mistakes prior the analysis. The automated protocol will be paused if the sample, reagent, or consumables are missing. Even during automated operations, pipette tip attachment/detachment and the column grip are monitored by sensors, preventing malfunctions.



Verification of positioning by image recognition

Detection of pipette tip attachment/detachment

Detection of grip errors

Quality Control Support Functions

The control software of the MUP-3100 automatically generates pretreatment results reports and stores them in the database along with pretreatment conditions. The MUP-3100 continuously monitors its operating performance, and if the device detects an error, its internal camera automatically creates a video of the 5 seconds before and after the occurrence, which is then saved along with error logs. This assists in identifying the root cause of errors and minimizing downtime.

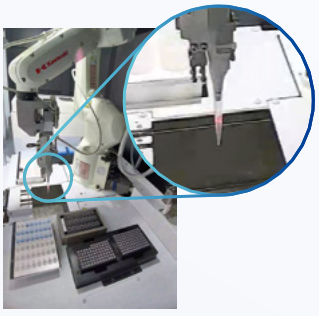
Abnormal condition

Automatically saves a video of the 5 seconds before and after detecting that the tip is not installed with a laser.

5 seconds ····· Abnormal condition ····· 5 seconds later

Normal condition

Verify the presence of the tip with a laser.



Processes 24 Culture Supernatant Samples Reliably in 6 Hours

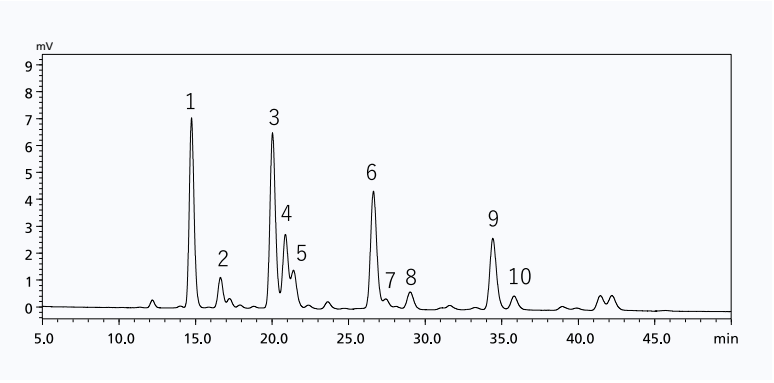
Complicated procedures can be automatically processed in 6 hours

Conventional methods require complex operations to cut out glycans from antibody samples in the culture supernatant. The traditional method requires more than two days for preprocessing. When using the MUP-3100 and the antibody *N*-glycan analysis kit in combination, however, 24 samples can be preprocessed in six hours.
Glycan profiling, including the type and quantity of glycans, can be performed by analysis of 2-AB labeled glycans using an HPLC fluorescence detector. The Shim-pack GIST-HP Amide [Metal Free] column is effective for separating out impurities and glycans contained in antibody preparations and culture supernatants.

MUP-3100

High performance liquid chromatograph (HPLC)

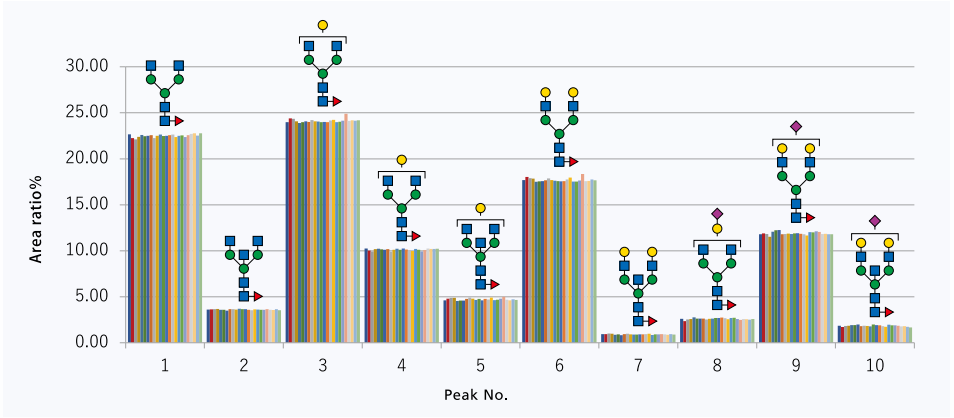
Liquid chromatograph mass spectrometer (LC-MS)



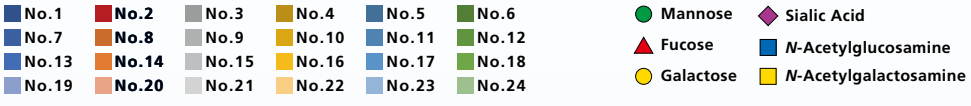
HPLC chromatogram for standard samples of antibody-derived glycans (from human serum IgG) with Shim-pack GIST-HP Amide [Metal Free] column

Allowing simultaneous processing of up to 24 samples

The MUP-3100 can process 24 samples at a time. Even with simultaneous processing of other samples, the area ratio of each peak remains stable regardless of peak intensity, yielding data equivalent to that obtained when using the kit manually.



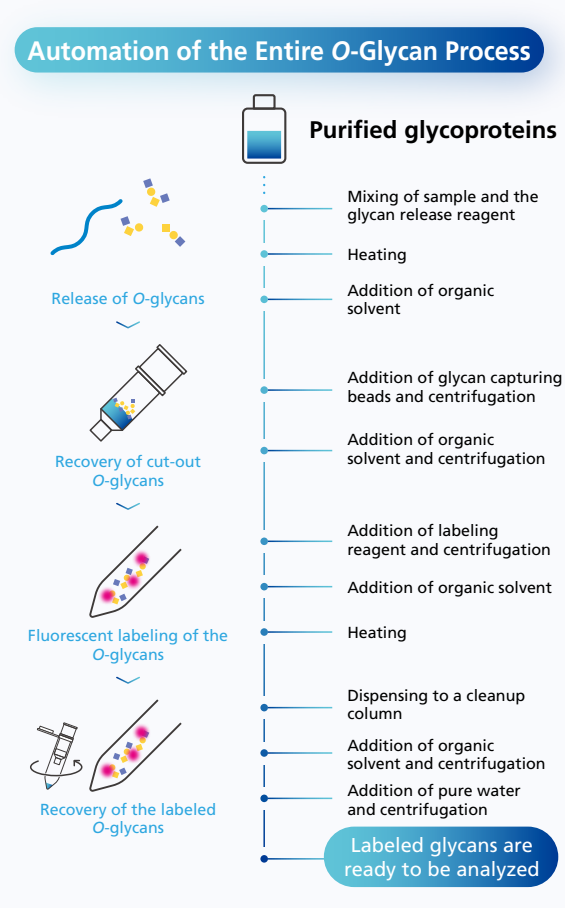
Comparison of peak area ratios for the *N*-Glycan (identical samples) derived from IgG in human serum



Example of the antibody *N*-glycan application data.

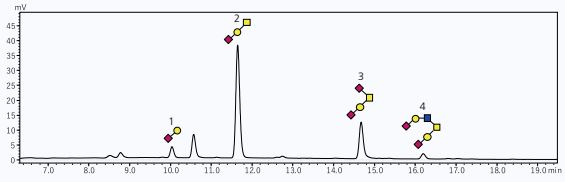
Application

O-Glycan Upgrade Kit

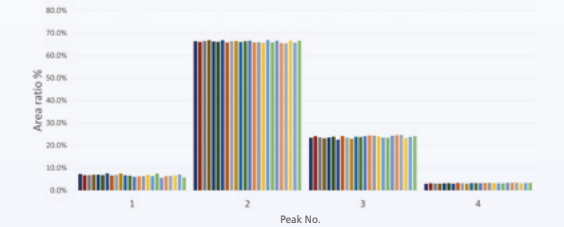


Stable Peak Profiles (MUP-3100 + *O*-Glycan Analysis Kit)

The MUP-3100 can accommodate 24 *O*-glycan samples at a time. Each peak area ratio is stable regardless of the peak intensity, even in multisample simultaneous analysis, so the data obtained is equivalent to when the *O*-glycan analysis kit is used manually.



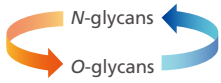
HPLC chromatogram for the analysis of *O*-linked glycans derived from bovine fetuin with the Shim-pack GIST-HP Amide metal-free column



Comparison of peak areas for *O*-glycans derived from bovine fetuin (same sample)

Addition of Pretreatment Functions for *O*-Glycans to the MUP-3100 (MUP-3100 + *O*-Glycan Analysis Kit)

Introducing the *O*-glycan upgrade kit (*O*-Type Reagent Tray + S/W License) enables the pretreatment of *O*-glycans as well as *N*-glycans.
The pretreatment of *O*-glycans using conventional methods requires two days, and is a hazardous process involving the use of anhydrous hydrazine, which is toxic. With this option, however, processing can be done safely in 11 hours. As with *N*-glycans, glycan profiling, including the type and quantity of glycans bound to the glycoproteins, can be performed by analysis of 2-AB labeled glycans using an HPLC fluorescence detector.
Switching between *N*-glycans and *O*-glycans can easily be implemented in the software program, enabling a flexible workflow, such as conducting pretreatment of *N*-glycans during the day, and *O*-glycans at night.



O-Glycan Analysis Kit (Sumitomo Bakelite Co., Ltd.) [*1] Auto-EZGlyco™ O-Glycan Prep Kit for SHIMADZU

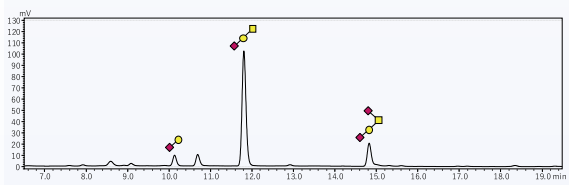
This enables everything from cutting out the *O*-glycans from the glycoprotein samples to purification and fluorescent labeling. Releasing the glycans by eliminative oximation[*2] minimizes peeling while providing a high recovery rate. This kit is effective for glycoprotein *O*-glycans other than antibodies.

Product

[*1] This kit is designed for automated systems. A manual kit is also available separately, so please contact us for more information.
[*2] Kameyama et al., A practical method of liberating *O*-linked glycans from glycoproteins using hydroxylamine and an organic superbase
Biochemical and Biophysical Research Communications, 513 (1), pp. 186-192 (2019)

Example of the Application of the *O*-Glycan Analysis Kit (Pretreatment Using the MUP-3100)

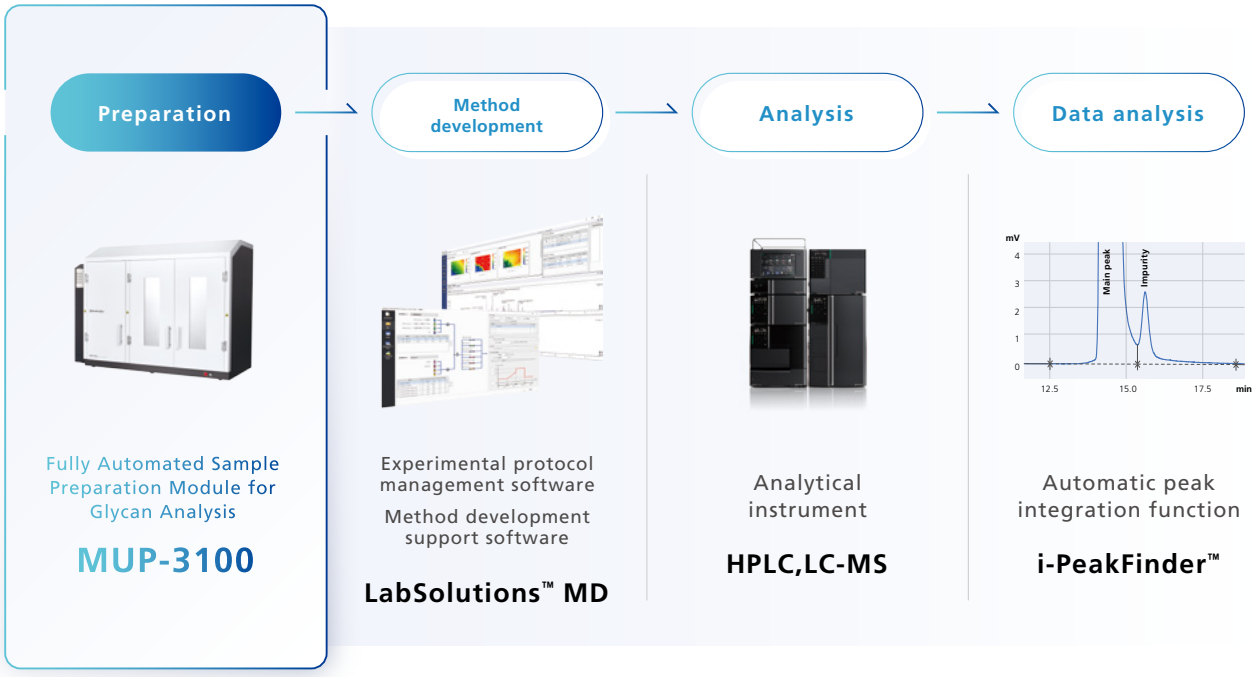
Etanercept is a type of antibody pharmaceutical, a fusion protein consisting of TNF receptors and the constant region of immunoglobulin IgG1. In addition to *N*-glycans, it has a number of *O*-glycans, and its structure is known to have an impact on the half-life in blood and an affinity with TNFa. *O*-glycans can be analyzed by combining the *O*-glycan analysis kit with the MUP-3100, a combination that enables the analysis of *O*-glycans on various glycoproteins, not just antibodies.



HPLC chromatogram of *O*-linked glycans derived from etanercept analyzed using the Shim-pack GIST-HP Amide metal-free column

Total Analytical Solutions

Shimadzu offers automation and labor-saving procedures from test planning to data analysis as total analytical solutions, with AI technology providing excellent data reproducibility.



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