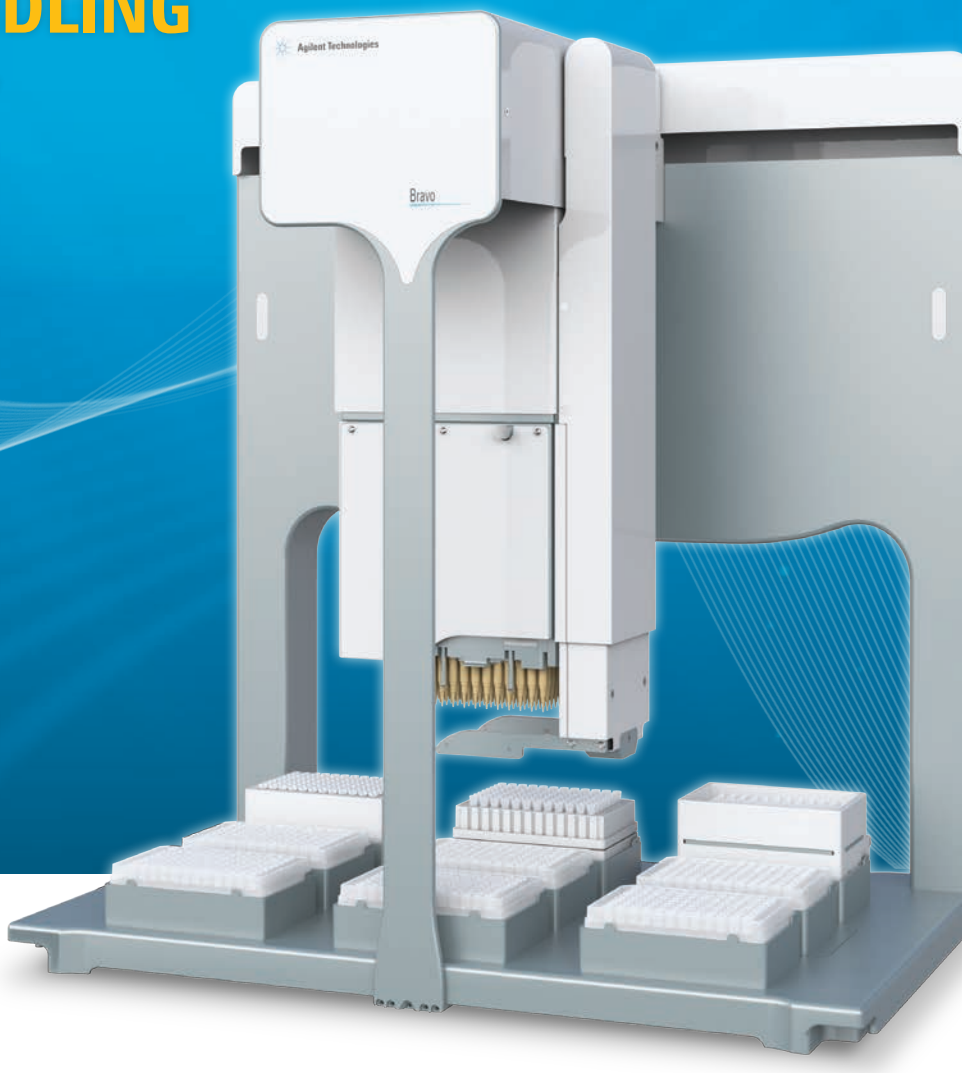
Agilent Bravo Automated Liquid Handling Platform

LIQUID HANDLING MADE EASY

The Measure of Confidence



Agilent Technologies

BOOST PRODUCTIVITY AND INCREASE CONFIDENCE IN YOUR RESULTS

The fastest, smallest, most versatile liquid handling system available.

Agilent's Bravo Automated Liquid Handling Platform provides accurate and precise pipetting over a wide volume range to improve your data quality and consistency. It has the flexibility and scalability to provide an extensive choice of configurations, and a unique open design that facilitates integration into additional workflows in your lab. Incorporating the Bravo platform into your research will free you from the hours spent manually setting up and running complicated applications.

Automate your critical applications:

- Pharmacodynamics
- Peptide mapping
- qPCR and PCR preparation and purification
- Protein purification
- Protein biomarker verification and validata
- MALDI sample preparation
- Compound library management
- Genomics, including microarray setup, SNPs and NGS

Find out how Bravo can automate these applications on the following pages.

Available in two space-saving models

The Bravo Automated Liquid Handling Platform is available in two models. The Bravo fits most in standard laminar hoods without disrupting airflow, while the Bravo SRT is three inches shorter to accommodate smaller hoods. Both enable automated liquid handling for cell-based assays or hazardous reagent handling.

Agilent VWorks Automation Control software

The VWorks software features an intuitive graphical user interface that makes it easy for users with all levels of experience to create new protocols, connect and configure devices, run protocols, and monitor progress. The built-in database is capable of managing most labware, including standard microplates, filter plates, deep well microplates, and tip boxes.

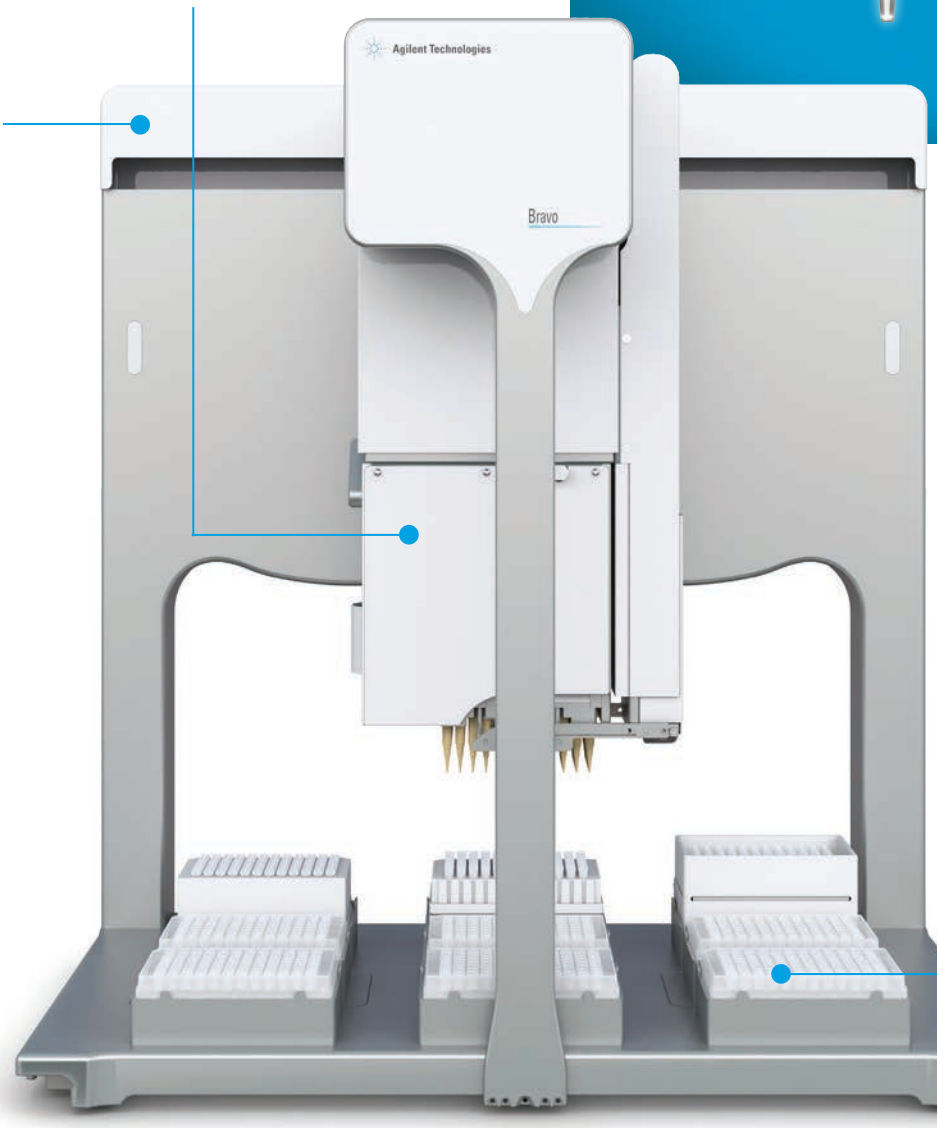
AssayMAP[1] Bravo Microchromatography Platform

Combines the precise and versatile Agilent Bravo liquid handler with AssayMAP microchromatography probe syringe technology from BioSystem Development. It incorporates a cartridge with a 5 µL bed of resin that functions as a miniaturized chromatography column and is coupled directly to small syringes in the head of the Bravo liquid handler for very precise flow rate control.

[1] *AssayMAP is a registered US trademark of BioSystem Development*

High-accuracy pipette heads

4 different: LT, ST, Assay Map, Fixed tip 50uL easy-change, high-accuracy pipette head types for dispensing from 100 nL to 250 µL in 96-, 384, and 1536-well formats, to help provide the highest throughput in the smallest footprint.



9-deck positions

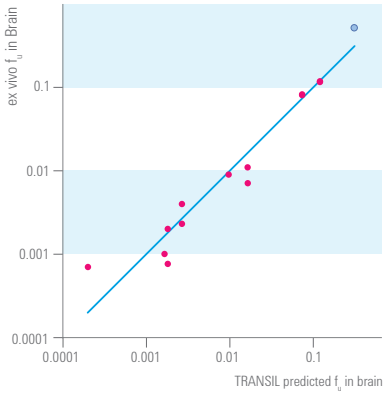
Versatility is maximized with 9 microplate positions on the deck, each of which can be configured for shaking, heating, cooling, filtration and more. ANSI-compliant microplates and reservoirs, in 96, 384 and 1536-well formats can be accommodated. A unique open design pipetting deck allows for access from all sides and simple system integration.

On-deck accessories

A wide range of sample preparation, assay setup and processing tasks can be enabled with the accessories that sit on the deck positions, including a filtration station, heating and cooling stations, an orbital shaker and wash, autofilling and weigh stations.

INCREASE YOUR PACE OF DISCOVERY WITH AUTOMATED WORKFLOWS

PHARMACODYNAMICS: Quickly and reliably determine blood-brain transfer and brain tissue binding of drug targets



The free fraction of drugs in the brain (fu) determined by the TRANSIL Brain Absorption kit using the automated protocol correlates strongly (R2=0.94) with the free fraction estimate determined by dialysis against brain homogenate.

The Bravo Automated Liquid Handling Platform has been utilized to automate an innovative, fast and simple blood-brain barrier assay to link brain penetration to pharmacodynamics. The TRANSIL Brain Absorption kit from Sovicell determines the affinity of drug candidates for the brain's membrane, and hence the free fraction of drug candidates in the brain.

Screen large numbers of non-CNS (CNS-) compounds for those that are less likely to cause undesirable side effects in the brain.

- 96-well format using the Agilent Bravo Automated Liquid Handling Platform, the Agilent Automated Microplate Centrifuge, and LC/MS
- The amount of each drug in the free fraction was determined
- All of the drug classifications (CNS+/CNS-) matched classifications by other methods and were identical to results obtained by manual use of the kit

Sovicell's TRANSIL Brain Absorption kit on Agilent's Bravo Liquid Handling Platform: an innovative, fast, simple bloodbrain barrier assay to link brain penetration to pharmacodynamics. **Agilent Application Note 5990-6628EN**

PEPTIDE MAPPING: Automated MALDI MS sample preparation for rapid determination of accurate and reliable results

Purification method	Sample preparation	Mascot score	Protein sequence coverage
VeloTips	Automatic	308	49%
Milipore ZipTips	Manual	248	39%
No Purification	Manual	127	29%

Comparison of sample preparation methods

High quality peptide mass fingerprints in MALDI mass spectrometry require concentrated peptides free from impurities introduced during digestion. Automation of chromatographic cleanup methods has never been truly feasible due to technical limitations such as imprecise liquid handling.

Use the unique Glygen VeloTip and with the Bravo Automated Liquid Handling Platform to automate your MALDI cleanup workflow.

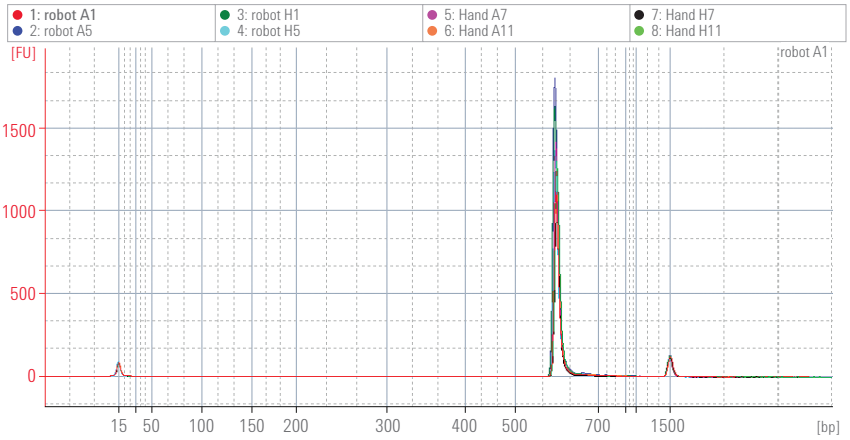
- C18 resin reliably concentrates even small amounts of low abundance peptides
- Binding, washing, elution, and direct spotting onto MALDI targets are all done automatically
- Results with trypsin-digested bovine serum albumin are comparable to those obtained with manual methods

Automated sample preparation and spotting on MALDI target plates using the Agilent Bravo Automated Liquid Handling Platform. **Agilent Application Note 5990-6241EN**

PCR: Automate setup and purification for results you can count on

The Bravo Automated Liquid Handling Platform is an effective tool to speed setup of a variety of quantitative polymerase chain reaction (qPCR) assays, while maintaining reliable results. For example, qPCR reactions to examine a variety of housekeeping genes from cDNAs derived from different human tissues were set up on the Bravo platform as well as by hand. Dilutions performed by each method showed similar slopes and good linearity, and targets amplified by both methods gave very similar Ct values.

Automation of PCR purification in a 96-well format using the Agilent Bravo Automated Liquid Handling Platform and Automated Centrifuge as well as the StrataPrep 96 PCR Purification Kit eliminates the need for tedious manipulation of resins, toxic phenol–chloroform extraction, and time-consuming ethanol precipitation. Electrophoretic analysis on the Agilent 2100 Bioanalyzer showed identical sizing and purity for both hand and robot-purified PCR products. A protocol has also been developed to automate the Millipore Montage PCR purification procedure.



Co-migration of manual and robot-purified PCR products. Note the absence of contaminating peaks and the comparable relative abundance of the product.

Complete Automation of the Stratagene StrataPrep 96 PCR Purification Kit with the Agilent Bravo Automated Liquid Handling Platform and Agilent Automated Centrifuge. **Agilent Application Note 5990-3948EN**

QPCR: Quantitative polymerase chain reaction studies

The Bravo Automated Liquid Handling Platform has been shown to be an effective tool to speed setup of a variety of quantitative polymerase chain reaction (qPCR) assays, while maintaining reliable results. For example, qPCR reactions to examine a variety of housekeeping genes from cDNAs derived from different human tissues were set up on the Bravo platform as well as by hand. Dilutions performed by each method showed similar slopes and good linearity, and targets amplified by both methods gave very similar Ct values.

Target	Fetal Lung			HeLa			Fetal Heart		
	Bravo	Hand	D	Bravo	Hand	D	Bravo	Hand	D
ACTB	20.37	20.04	0.33	19.87	20.09	0.22	19.52	19.54	0.02
B2M	23.86	23.6	0.26	22.64	22.93	0.29	20.21	20.15	0.06
GAPDH	22.74	22.44	0.3	19.28	19.36	0.08	18.6	18.43	0.17
GUSB	26.46	26.64	0.18	25.12	25.18	0.06	25.32	25.27	0.05
HPRT1	29.77	29.91	0.14	25.86	26.33	0.47	25.92	25.79	0.13
PGK	26.47	26.35	0.12	22.39	22.61	0.22	22.77	22.62	0.15
PP1A	22.53	22.28	0.25	20.94	21.37	0.43	20.5	20.44	0.06
RPL13A	20.67	20.13	0.54	20.21	20.36	0.15	18.77	18.55	0.22
TBP	28.85	29.03	0.18	28.55	28.85	0.3	27.85	28.11	0.26
TFRC	29.51	29.71	0.2	24.66	24.91	0.25	27.28	27.4	0.12
ß-actin	20.78	20.83	0.05	20.53	20.84	0.31	17.52	17.75	0.23

Comparison of Ct values obtained when preparing targets using the Bravo or by hand.

Cytochrome P450 Assay on the Agilent Bravo Automated Liquid Handling Platform.
Agilent Application Note 5990-3550EN

AUTOMATED SOLUTIONS FOR A RANGE OF PROTEIN ANALYSIS CHALLENGES

Validate your biomarkers to a high level of confidence

The challenge

Providing the sensitivity and throughput required to meet the demands of biomarker validation in large numbers of extremely complex samples.

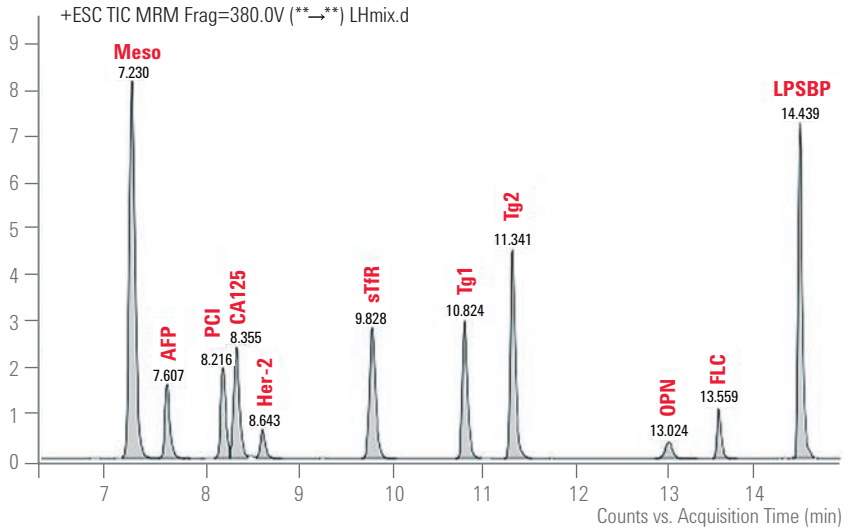
The solution

SISCAPA Automation on the Bravo Platform - process samples in less than half the time required by traditional manual methods!

In recent years, biomarkers have become one of the major drivers in pharmaceutical research and drug development, as they can be used to measure disease risk or progression and potential effectiveness of certain treatments, serve as targets for drug discovery, provide evidence in support of go/no-go decisions at drug development phase gates, and enable development of clinical tests for targeted therapeutics.

SISCAPA† technology (Stable Isotope Standards and Capture by Anti-Peptide Antibodies), can enrich low abundance biomarkers >100,000-fold in complex samples such as serum, using anti-peptide antibodies to enrich for the signature peptides of putative biomarkers, prior to MRM analysis. Both sensitivity and specificity are enhanced while preserving quantitative information throughout capture and elution of the peptides.

Automation of a SISCAPA Magnetic Bead Workflow for Protein Biomarker Quantitation by Mass Spectrometry Using the Agilent Bravo Automated Liquid Handling Platform. **Agilent Application Note 5990-7360EN**



Peaks in the LC chromatogram produced by the best transition for each of the 11 peptides monitored in the SISCAPA experiment.

"We have found the Bravo very effective in automating magnetic bead-based affinity capture of peptides for mass spectrometry as well as a range of associated precision liquid handling tasks. The new AssayMAP technology has tremendous potential for further accelerating the SISCAPA methodology"

- **LEIGH ANDERSON,** ANDERSON FORSCHUNG GROUP, WASHINGTON, D.C. USA

Automate your protein purification for superior results

The challenge

Efficiently automate reliable high-throughput purification of protein samples without excessive reagent use.

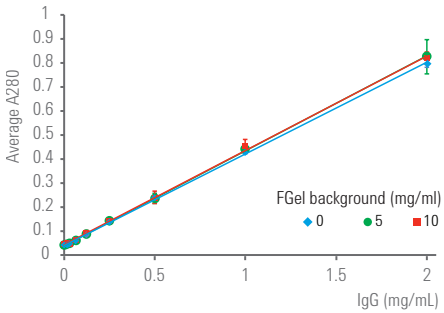
The solution

The Agilent AssayMAP Bravo platform with AssayMAP microchromatography delivers rapid, linear, and highly reproducible recovery of proteins from complex samples.

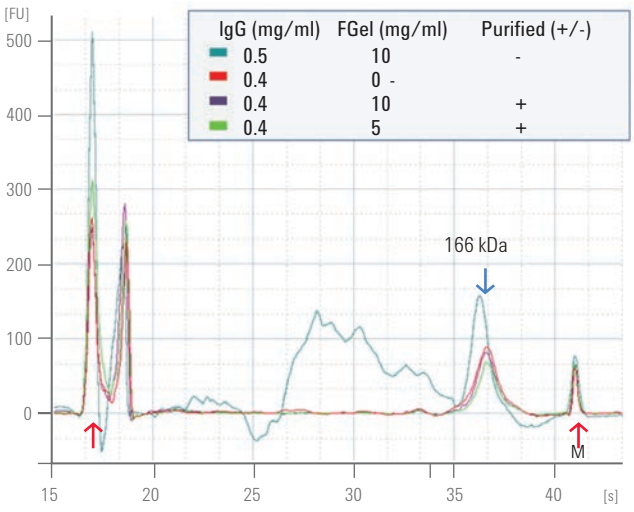
The increasing importance of biotherapeutics in recent years has caused an increase in demand for high precision, high sensitivity and high throughput protein analysis techniques. Chromatography based methods have long been the gold standard to purify specific proteins from complex samples, but these methods have not been adapted to the requirements of high throughput analysis.

The capabilities of the AssayMAP Bravo platform with 96-channel liquid handling head have been demonstrated by the affinity purification of immunoglobulin G (IgG) antibodies, using immobilized Protein A. Linearity of recovery is nearly perfect across a 1 to 100 µg range of human immunoglobulin G (hIgG), even in the presence of as much as 10mg/mL of background protein. The superimposed output from the analysis of four different samples containing varying amounts of background protein illustrates the high degree of purification. In addition to Protein A, the microchromatography cartridges can be packed with polymeric, agarose, or silica-based materials in all of the common chromatographic modes for a wide variety of high throughput protein purification applications.

High throughput purification of human IgG using the Agilent Bravo for Protein Purification and AssayMAP protein A cartridges. **Agilent Application Note 5990-7203E**



Absorbance values of eluates from Protein A cartridges loaded with hIgG and different concentrations of fish gelatin (FGel) protein. R2 values for the three different sample sets of 0, 5, and 10 mg/mL background protein were 0.9997, 0.9998, and 0.9992, respectively.



Superimposed electropherogram traces from Bioanalyzer Protein 230 chips. The 166 kDa peak indicates the expected position for hIgG.

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