



Biomarker Workflow Solutions

A toolbox for successful biomarker discovery, development, and validation



FROM DISCOVERY TO VALIDATION

Biomarkers provide vital information regarding safety and efficacy during drug discovery and development. They can also be used to select patients for clinical trials, to monitor their response, and to monitor a treatment's efficacy. In addition, after regulatory approval, biomarkers can be used in the clinic to allow more personalized treatments.

Biomarkers range from specific molecules such as a protein, gene sequence, or expressed RNA to physiological parameters such as blood pressure or body temperature. They may be expressed in a specific location or tissue or found in serum, plasma, and other bodily fluids. Because of this variety most successful biomarker programs have a broad reach and investigate cellular, proteomic, and genetic sources in order to find the most valuable biomarkers. Bio-Rad's broad product portfolio is able to support biomarker discovery across all of these areas and is ideally suited to support even the most comprehensive biomarker programs.

BIO-RAD'S BIOMARKER WORKFLOW: FROM DISCOVERY TO VALIDATION

CELLULAR



ZE5™ CELL ANALYZER



S3e™ CELL SORTER



ZOE™ CELL IMAGER

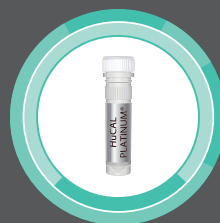
PROTEOMIC



V3 WESTERN WORKFLOW™



BIO-PLEX® 200 SYSTEM



HuCAL® CUSTOM
ANTIBODY TECHNOLOGY

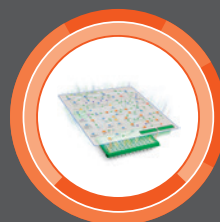
GENOMIC



QX200™ DROPLET
DIGITAL™ PCR SYSTEM



CFX AUTOMATION
SYSTEM II



PrimePCR™
PANELS

SOLUTIONS BEYOND DISCOVERY



BIO-PLEX® 2200 SYSTEM



QUALITY CONTROLS



DIAGNOSTIC ANTIBODIES

CELLULAR BIOMARKERS

UNCOVER RARE CELL POPULATIONS



ZE5™ CELL ANALYZER



S3e™ CELL SORTER

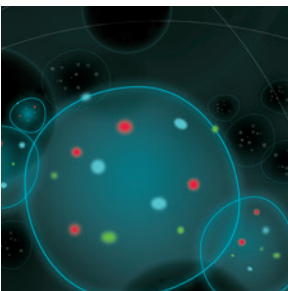


ZOE™ CELL IMAGER

The ability to uncover novel and rare cell types through multi-parameter analysis allows researchers to characterize cell populations in greater detail and identify subpopulations that can serve as markers for disease. Simple and reliable isolation of these cells for downstream assays further supports the cellular biomarker workflow by providing pure populations for genomic and proteomic analysis.

Our comprehensive and easy-to-use suite of cell analysis products includes the ZE5 Cell Analyzer, the S3e Cell Sorter, and the ZOE Fluorescent Cell Imager, as well as a wide range of flow cytometry and cell imaging reagents. Examine up to 30 parameters simultaneously with the ZE5 Cell Analyzer to distinguish subtly different cell populations, sort rare and single cells with the S3e Cell Sorter, and perform traditional immunocytochemistry and -histochemistry studies for biomarkers in live or fixed cells right at your bench with the ZOE Cell Imager.

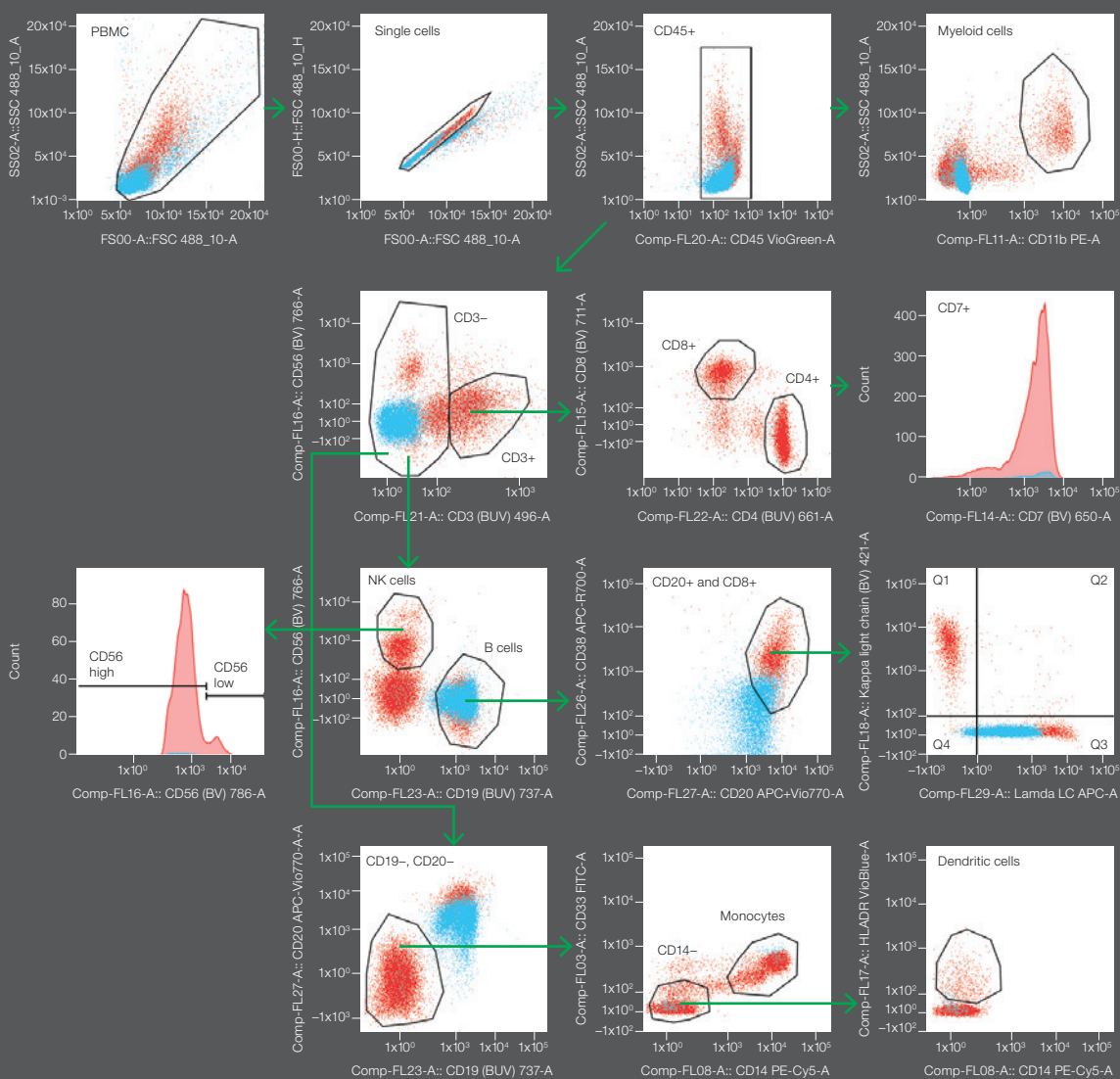
In this example we demonstrate the ability of the ZE5 Cell Analyzer to allow broad yet in-depth analysis of a blood sample in a single run. A 15-color panel of known leukocyte markers was used to differentiate subpopulations of normal peripheral blood mononuclear cells (PBMCs) and those from a patient with chronic B cell leukemia.



SUCCESS STORY Bio-Rad bulletin 6950 shows how the ZE5 Cell Analyzer can be used to design and optimize a 21-color panel for quantification of T cell subsets in stem cell transplant patients.

Visit bio-rad.com/biomarker1 to learn more.

15-COLOR CELLULAR BIOMARKER SCREENING PANEL FOR CHRONIC B CELL LEUKEMIA



Differentiating subsets of malignant cells from normal cells using a 15-color panel of known leukocyte markers. We were able to distinguish and define a broad range of immune cell phenotypes within normal and chronic B-cell leukemic PBMC samples. Our 15-color panel provides the ability to separate PBMC into base cell populations and includes broad prognostic and diagnostic markers such as CD7, CD38 kappa, and lambda light chain. Normal and diseased PBMCs (AllCells.com) were thawed and allowed to recover in culture media at 37°C, 5% CO₂ for at least one hour. Single-color antibodies were pooled, mixed gently with one drop of AbC Total Antibody Compensation Beads (Thermo Fisher), and incubated for at least one hour. Unstained controls, normal, and diseased PBMCs were incubated in blocking buffer for 45 min on ice before staining with the 15-color antibody cocktails for 1 hour. Cells and beads were spun down, washed once in wash buffer, and then resuspended and analyzed on the ZE5 Cell Analyzer. Positive and negative bead populations were used to set compensation for fluorochromes. ZE5 Cell Analyzer parameters: 5 lasers at 355 nm, 405 nm, 488 nm, 561 nm, and 640 nm; fluorescence detectors and dual forward-scatter channels at 405 nm and 488 nm and a side scatter channel.

PROTEOMIC BIOMARKERS

MULTIPLEX YOUR PROTEIN ANALYSIS



V3 WESTERN WORKFLOW™



BIO-PLEX® 200 SYSTEM



HuCAL® ANTIBODY GENERATION TECHNOLOGY

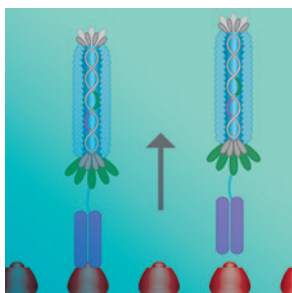
Interrogating your sample to identify its proteomic profile gives rise to a veritable feast of potential biomarkers. In addition, monitoring the posttranslational state of proteins of interest can yield important biomarkers.

The V3 Western Workflow is a validated and streamlined workflow that allows quantitation of protein expression faster and with more accuracy than other western blotting procedures. By incorporating the ChemiDoc™ MP Imaging System, this workflow allows simultaneous analysis of up to three proteins via multiplex fluorescent western blotting.

Bio-Plex Multiplex Immunoassay Systems and Assays allow analysis of large numbers of biomarkers in a single experiment. Predesigned panels for a given indication remove the guesswork from your search while custom assays allow you to design panels for your unique markers as your biomarker program progresses.

Antibody selection and antibody specificity are crucial to the performance of your proteomic assays. Our HuCAL custom antibody technology can generate highly specific custom human antibodies in only 8 weeks for a variety of applications, including flow cytometry, ELISA, and western blotting.

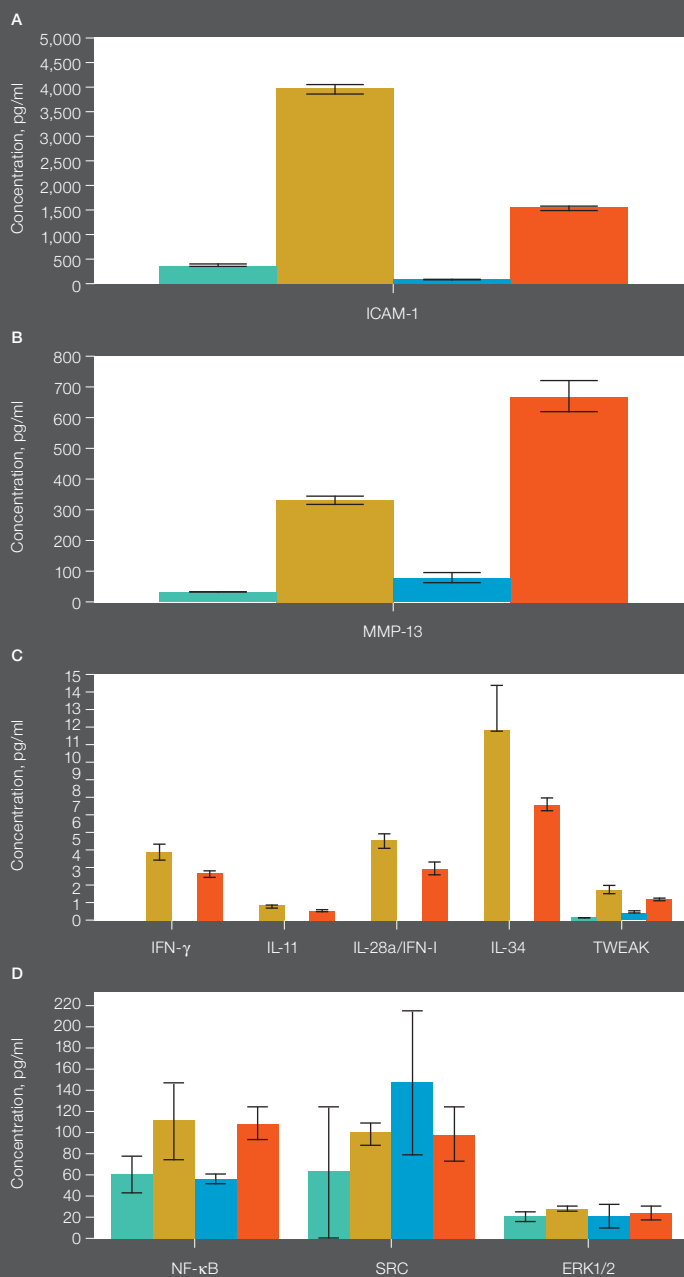
For this example we identified proteins whose expression is modulated by TNF- α in cancer cell lines, we investigated changes in protein expression in wild type and knock out prostate cancer cell lines with and without TNF- α stimulation.



SUCCESS STORY Evidence suggests that CXCL10 is a strong predictor of poor clinical outcomes from HCV treatment. We show that HuCAL Antibodies can quantitatively discriminate between agonist and antagonist forms of CXCL10, which differ by only two amino acids at the N-terminus.

Visit bio-rad.com/biomarker2 to learn more.

PROTEIN BIOMARKER DISCOVERY USING MULTIPLEX IMMUNOASSAYS



Measuring protein expression in wild-type PC3 cancer cells and *ICAM-1* knock down PC3 cells using Bio-Plex Multiplex Immunoassays.

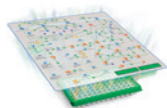
Cell lysates were collected from each of the four sample types after *ICAM-1* induction with TNF- α . We found that **A**, *ICAM-1* knock down is incomplete; *ICAM-1* expression is still induced, albeit at a lower level (Bio-Plex Pro Human Cytokine 21-Plex Panel, catalog #MF005KMII) and that **B**, *MMP-13* expression is 2-fold higher in the edited PC3 cell line than in wild-type PC3 cells (9-plex Bio-Plex Pro™ Human MMP Panel, catalog #171AM001M). We also observed **C**, attenuated cytokine induction in the edited PC3 cell line in response to TNF- α (37-plex Bio-Plex Pro Human Inflammation Panel 1, catalog #171AL001M); and **D**, found that three cytokines, p-Src, p-NF- κ B and p-Erk1/2, were detectable in both wild-type and edited PC3 cell lines but expression levels remained unchanged upon TNF- α stimulation (Bio-Plex Pro Magnetic Cell Signaling Assays, various catalog numbers). Lysates were treated according to the instructions provided with each Bio-Plex Panel. PC3 (■); PC3 + TNF- α (■); edited PC3 (■); edited PC3 + TNF- α (■).

GENOMIC BIOMARKERS

DISCOVER MORE



CFX REAL-TIME PCR
DETECTION SYSTEM



PrimePCR™ PANELS



QX200™ DROPLET DIGITAL™
PCR SYSTEM

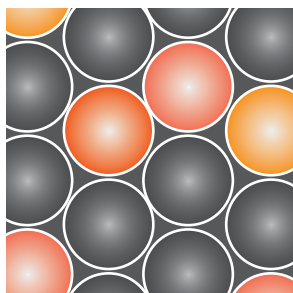


CFX AUTOMATION SYSTEM II

Both gene expression and the presence of specific genetic sequences are used as biomarkers for many diseases, including cancer. Treatments can even be tailored to the individual based on these genomic biomarkers. Typically, large-scale gene expression studies are conducted to identify genes that are up- or downregulated in a particular indication or disease. These studies rely on the ability of the researchers to design exploratory and confirmatory panels as well as on powerful software tools to analyze resulting data and identify potential biomarkers.

CFX Real-Time PCR Detection Systems along with custom-built or predesigned pathway or disease state PrimePCR Panels allow you to measure multiple genes from multiple samples at once, be they liquid biopsy, tissue, or cell-based materials. Coupled with the CFX Automation System II, up to 48 plates can be read unattended. If increased sensitivity is required, such as for the detection and quantification of CTCs and rare cancer mutations, the QX200 Droplet Digital PCR (ddPCR™) System is an ideal choice, as it allows detection of rare, low-abundance genetic biomarkers. Both systems are provided with IQ/OQ workflows and tools that enable 21 CFR Part 11 compliance.

In this example gene expression patterns from prostate cancer samples across four different stages were analyzed by quantitative PCR. Assay design allowed the reproducibility of the multiplex assay to be compared to singleplex analysis. After hierarchical clustering analysis and one-way ANOVA, potential candidate biomarkers were selected for inclusion in a five-color PrimePCR Multiplex Assay using CFX Maestro™ Software.



SUCCESS STORY Prospective studies have used ddPCR technology to quantify driver and resistance mutations associated with tumors such as melanoma and have reported positive predictive values of up to 100%, turnaround times of 2–3 days, and a significant increase in sensitivity. ddPCR assays for metastatic melanoma have been found to be 42% more sensitive than traditional LDH assays (Chang et al. 2016).

Chang et al. (2016). Sensitivity of plasma *BRAF* mutant and *NRAS* mutant cell-free DNA assays to detect metastatic melanoma in patients with low RECIST scores and non-RECIST disease progression. *Molecular Oncology* 10, 157–165.

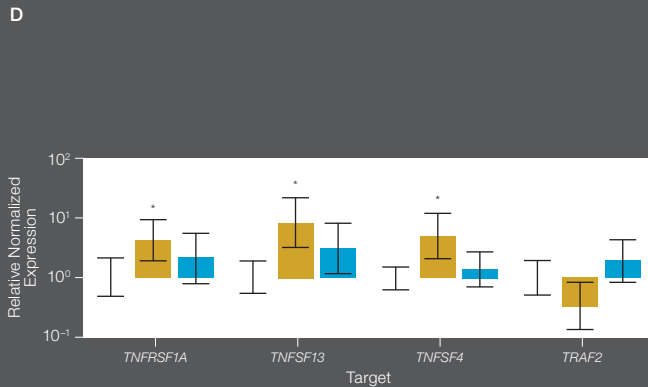
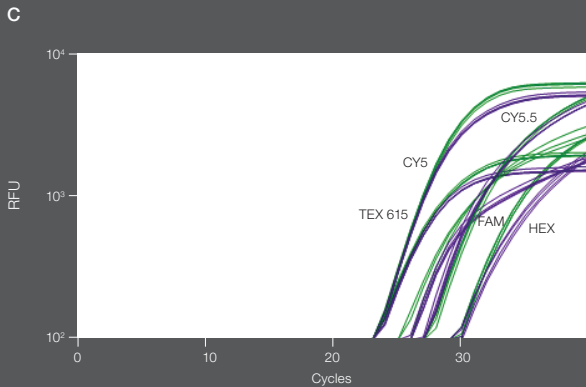
GENETIC BIOMARKER DISCOVERY USING HIGH THROUGHPUT REAL-TIME PCR



B

ANOVA Results	
Target	P Value
<i>TNFSF13</i>	0.022585
<i>TNFSF4</i>	0.011183
<i>TNFRSF1A</i>	0.177595
<i>TRAF2</i>	0.017002

Tukey HSD, P Value			
Contrast	<i>TNFSF13</i>	<i>TNFSF4</i>	<i>TRAF2</i>
Normal – Stage II	0.013433	0.018559	0.230739
Normal – Stage III	0.344713	0.927900	0.675594
Stage II – Stage III	0.367151	0.048767	0.013329



Discovering prostate cancer biomarkers using gene expression analysis. **A**, CFX Maestro Software for CFX Real-Time PCR Instruments allows data to be analyzed in clustergram by sample, by target, or both (subset of data shown); normal, (●); stage II, (●); stage III, (●); stage IV, (●); **B**, One-way ANOVA of gene expression in biological groups (stages of prostate cancer). The ANOVA *P* values indicate that there was a statistically significant difference among the means, which was further supported by the Tukey HSD test. Statistically significant differences are noted with *P* values in red. Based on ANOVA of all targets, *TNFSF4* and *TRAF2* may be useful in distinguishing stage II from stage III prostate cancer, while *TNFSF13* and *TNFSF4* may be useful in distinguishing prostate cancer from normal cells; **C**, Validation of five-target multiplex assay composed of five unique PrimePCR Probe Assays. Multiplex (■) versus singleplex (■) traces; targets: *TRAF2* (FAM), *TNFSF13* (HEX), *TNFSF4* (TEX 615), *HPRT1* (CY5), *TNFRSF1A* (CY5.5). Pre-amplified cDNA from normal prostate tissue shown; **D**, Normalized gene expression by biological group (stages of prostate cancer) was averaged. Error bars are 95% confidence intervals. Number of samples: 16 normal, 20 stage II, 22 stage III. Based on these results, *TNFRSF1A* does not seem to be a useful biomarker for prostate cancer progression. Normal (■); stage II (■); stage III (■); *, significant difference compared to the signal from the normal sample.

SOLUTIONS BEYOND DISCOVERY

TOOLS TO TAKE TO THE CLINIC



BIOPLEX® 2200 SYSTEM



QUALITY CONTROLS



DIAGNOSTIC ANTIBODIES



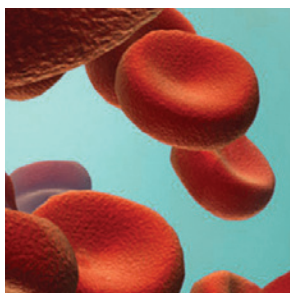
HuCAL® ANTIBODY
GENERATION TECHNOLOGY

Our broad range of solutions for gene, protein, and cellular biomarker discovery allows you to uncover markers for your specific needs. Each biomarker's proposed downstream application defines the extent to which it must be validated before it can move forward, to the clinic and beyond. Validation data include selectivity, specificity, accuracy, precision, reproducibility, and analyte stability. Whatever your validation pathway or downstream need, you must have access to a reliable stream of assay components and quality controls.

Bio-Rad is the market-leading manufacturer of high-quality antigens for use in biomarker and clinical diagnostic test development. Our cell culture antigens offer an uninterrupted, unlimited supply of consistent, stable, high-quality reagents. To accompany our antigens, we have developed monoclonal antibodies for use in the development of immunohistochemistry, western blotting, and other assay types. If a novel antibody is needed, our HuCAL® custom antibody generation service can generate a human antibody within 8 weeks using an animal-free platform.

Additionally, we provide human-based controls covering a wide variety of analytes at subtherapeutic, therapeutic, and elevated levels to monitor the precision of testing procedures in the clinic. If you are looking to incorporate testing for known biomarkers into a clinical trial, you can take advantage of our clinical diagnostic offerings, including ELISA- and HPLC-based instruments, and clinical diagnostic assays and kits that are registered for IVD use.

Bio-Rad partners with you on your biomarker journey, providing solutions from early discovery to the clinic and beyond, and meeting your needs for security of supply of critical materials. Now your journey is complete.



SUCCESS STORY Not all biomarker HbA1c tests are created equal. For over 40 years Bio-Rad has delivered gold standard HPLC technology to give customers like Biomed 21 accurate results plus variant detection. HbA1c methods that do not detect hemoglobin variants might lead to misdiagnosis and unnecessary or delayed patient treatment. Hear from Bernard Desprats about the D-100™ Smart HPLC System.

Visit bio-rad.com/biomarker3 to watch the video.

SOLUTIONS BEYOND DISCOVERY



WHY BIO-RAD?

Bio-Rad has over 60 years of experience supporting life science and clinical diagnostics. We are the leader in digital PCR, transfection, and in gene and protein expression analysis technologies. Our innovative products are available as stand-alone solutions or combined into workflows that are optimized to minimize downtime and to provide the right answer the first time, every time. Our Biomarker Workflow Solutions are one of

many Bio-Rad workflows designed to shorten discovery time and increase success rates for novel therapeutics. As part of our commitment to your research, and to increase the success rates for therapeutics and biosimilars, we provide:

- Products and reagents that are reliable and validated
- Responsive, trusted global service and technical support teams
- Technical expertise across multiple workflows and applications

Life Science RUO*	Discovery	Development	Validation
Cells			
S3e Cell Sorter	•	•	•
ZE5 Cell Analyzer	•	•	•
ZOE Fluorescent Cell Imager	•	•	•
TC20 Cell Counter	•	•	•
Antibodies for Flow Cytometry and Immunohistochemistry	•	•	•
Genes			
QX200 Droplet Digital PCR System	•	•	•
PrimePCR Assays for qPCR and ddPCR	•	•	•
CFX Real-Time PCR Detection Systems	•	•	•
qPCR Reagents and Consumables	•	•	•
Proteins			
Bio-Plex Multiplex Immunoassay Systems	•	•	•
Bio-Plex Multiplex Immunoassays	•	•	•
Stain-Free Mini and Midi Protein Gels	•	•	•
Trans-Blot® Turbo™ Transfer System	•	•	•
ChemIDoc MP Imaging System	•	•	•
PrecisionAb™ Validated Western Blotting Antibodies	•	•	•
Protein Sample Preparation Beads and Kits	•	•	•
HuCAL Generated Custom Antibodies	•	•	•

* Products listed in this table are for research use only.

In addition to an array of RUO products for use in your biomarker program, Bio-Rad has a broad selection of products that already meet regulatory requirements and can also be incorporated.¹ These products cover many applications: diabetes testing²; autoimmune testing²; immunohematology³; blood virus testing⁵ and quality controls⁵

¹ Availability differs depending on country and/or region. ² U.S. FDA 510(k) clearance. ³ U.S. FDA 510(k) clearance and U.S. FDA licensed products. ⁴ U.S. FDA approved and U.S. FDA licensed products. ⁵ U.S. FDA 510(k) clearance or exemption.

Visit bio-rad.com/web/biomarker4 to learn more about how our products align with your biomarker program requirements.

AbC is a trademark of Thermo Fisher Scientific. FAM is a trademark of Applera Corporation. Cy is a trademark of GE Healthcare Group. Texas Red is a trademark of Invitrogen Corporation. TEX is a trademark of DNA Technologies, Inc.

The Bio-Plex Suspension Array System includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.

Bio-Rad's thermal cyclers and real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

The QX200 Droplet Digital PCR System is covered by claims of U.S. patents, and/or pending U.S. and non-U.S. patent applications owned by or under license to Bio-Rad Laboratories, Inc. Purchase of the product includes a limited, non-transferable right under such intellectual property for use of the product for internal research purposes only. No rights are granted for diagnostic uses. No rights are granted for use of the product for commercial applications of any kind, including but not limited to manufacturing, quality control, or commercial services, such as contract services or fee for services. Information concerning a license for such uses can be obtained from Bio-Rad Laboratories. It is the responsibility of the purchaser/end user to acquire any additional intellectual property rights that may be required.



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Laboratories, Inc.**

Life Science
Group

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