Therapeutic monoclonal antibodies

Applications for qPCR in therapeutic monoclonal antibody development and manufacturing

Effective and reliable **Applied Biosystems™ QualTrak™ real-time quantitative PCR (qPCR) workflows** can help therapeutic monoclonal antibody (mAb) researchers and manufacturers incorporate genetic analysis of their investigational molecules into their processes. Performing genetic analysis at each stage from discovery through manufacturing can enable them to efficiently identify and quantify antigens, screen antibodies, identify antibody genes and biomarkers, develop and validate clones and cell lines, detect low levels of contamination, and help ensure the safety and efficacy of their products. The Applied Biosystems™ suite of real-time qPCR instruments, assays, and reagents can make it easier to navigate the critical steps in each stage of therapeutic mAb development and production from discovery through manufacturing. Implementing QualTrak workflows with strict quality control and the compliance documentation provided with Applied Biosystems™ TaqPath™ reagents and assays can expedite time-to-market. Thermo Fisher Scientific can provide the services and additional documentation that manufacturers need to achieve and maintain regulatory compliance.

Introduction

Monoclonal antibodies are immunoglobulins produced in a laboratory setting that bind to specific antigens, such as epitopes expressed on the surfaces of cells. They can be used to treat various diseases, including some forms of cancer [1]. Monoclonal antibodies are produced using hybridoma technology or the phage display technique [2]. With hybridoma technology, immortalized tumor cells are hybridized with mammalian B cells that produce antibodies. Hybridoma cells undergo multiple rounds of screening and selection, and cell lines that produce mAbs with the highest specificities for target antigens are chosen for mAb production. The phage display technique involves cloning a mAb gene construct into cells that will express the recombinant protein *in vitro*. The gene construct is carried into cells by a vector, such as a plasmid or virus. Stable cell lines that produce mAbs with the desired specificities are then selected for mAb production.

Real-time qPCR: an established genomic technology in the biopharmaceutical industry

The development and characterization of recombinant mAbs can be expedited by incorporating genetic analysis with qPCR at each stage. qPCR is an established industry standard for checking the quality of biologic drug development. Biopharmaceutical laboratories trust qPCR, know how to use it, and employ it for various applications throughout biopharmaceutical development. qPCR enables rapid and straightforward detection of targets. When it is performed correctly, laboratories can be confident they are obtaining accurate and reproducible results. Multiplex qPCR makes it possible to analyze more than one target in a single reaction, so it can provide results faster than single-plex qPCR. qPCR is also ideal for high-throughput workflows. Up to 96 or 384 samples can be analyzed in a single plate with a two-hour turnaround time. The wide dynamic range for targets in high and low concentrations makes qPCR flexible for various uses, and as few as 10 copies of a transcript can be detected in a single reaction well.

qPCR applications in the discovery phase

The discovery phase of therapeutic mAb development involves identifying a target antigen, screening antibodies for the target, identifying antibody genes, and identifying relevant biomarkers that indicate a phenotypic response to the mAb. qPCR is an indispensable and reliable technique for each of these use cases.

Use case: target antigen identification

Changes in gene expression in response to a pathogen or disease can be quantified with reverse-transcription qPCR (RT-qPCR) to identify antigenic targets for mAb therapeutics. The key feature of real-time gene expression analysis is the specificity of qPCR probes for the transcripts of interest. The probes in Applied Biosystems™ TaqMan™ assays are specifically designed to provide the high degree of specificity required for qPCR genotyping.

The specificity of qPCR genotyping also enables simple and specific clone validation during the development of constructs to express antigens *in vitro*. qPCR is a robust technique for traditional copy number variation (CNV) analysis. CNV analysis involves looking for multiple copies of a sequence in the genome, typically up to three. CNV or single-nucleotide polymorphism (SNP) analysis can be performed using qPCR to validate a gene of interest. Users can select Applied Biosystems™ qPCR master mixes and reagents that best fit their specific needs for genotyping and analyzing gene expression, CNVs, and SNPs. Applied Biosystems™ QuantStudio™ qPCR systems can provide 1.5-fold resolution, and up to five copies of a SNP can be resolved accurately and efficiently.

Use case: antibody screening and antibody gene identification

Gene expression analysis with RT-qPCR enables the detection of heavy chain and light chain gene expression *in vitro* for the production of functional recombinant mAbs. Real-time RT-qPCR allows genes for individual antibodies to be amplified and identified, even if they are expressed at low levels [3]. mAb libraries can then be screened to identify candidates that target specific epitopes. A **custom TaqMan gene expression assay** with specialty oligos or custom primers and probes can be used to validate a recombinant construct for expression of a mAb that has high specificity and strong affinity for the target epitope. Users can select qPCR master mixes and reagents that best fit their specific needs.

Use case: biomarker identification

Identifying relevant biomarkers is vital to bring the leading mAb candidate into the next phases of development and clinical trials. RT-qPCR is a user-friendly, sensitive, and specific tool for the accurate detection and quantitation of mRNA transcripts for biomarkers that may indicate disease or a phenotypic response to mAb therapy. For example, *HER2* genetic alterations have been associated with several types of cancer. qPCR is the most commonly used method to detect *HER2* mutations, *HER2* amplification, and *HER2* overexpression [4]. Monitoring prognostic genomic cancer biomarkers can be incorporated into clinical prognostic staging or practice guidelines.

Select the most promising mAb candidates by identifying sensitive and reliable therapeutic biomarkers *in vitro* early in the discovery phase. Performing microRNA (miRNA) analysis, SNP and CNV genotyping, and gene expression analysis with RT-qPCR can reveal suitable therapeutic targets, molecular pathways that lead to disease, and mechanisms of drug activity.

TaqMan master mixes

Applied Biosystems™ TaqMan™ master mixes for single-plex and multiplex real-time qPCR are compatible with a variety of qPCR platforms and deliver reliable, best-in-class performance. TaqMan real-time PCR master mixes provide exceptional lot-to-lot consistency and include passive reference dyes to control for common sources of variability like small fluorescence fluctuations, PCR inhibitors, and carryover-contamination control reagents.

TaqMan assays for mAb discovery

We offer more than 20 million highly specific, quantitative, and sensitive Applied Biosystems™ TaqMan™ 5′ nuclease assays for gene expression analysis that come with predesigned qPCR primers and probes. TaqMan gene expression assays for members of highly homologous gene families typically amplify targets at least 10 cycles earlier than the closest homologs and amplify with 1,000-fold discrimination or more when equal numbers of two targets are present. Our **predesigned TaqMan assays** are ideal for measuring low-level gene expression and low-abundance targets, and small changes in gene expression can be accurately quantified.

qPCR applications in the process development phase

In the process development phase, mAb manufacturers develop assays, tests, and standard operating procedures (SOPs) that must be rigorously followed during preclinical and clinical trials and at the manufacturing stage. Generating reproducible data with reliable tools is essential for maintaining genetic consistency and regulatory compliance throughout mAb cell line and master cell line bank process development. It is thus important to adhere to current good manufacturing process (cGMP) principles and ensure that a robust quality control (QC) system is in place with documentation from all suppliers involved in the mAb development process.

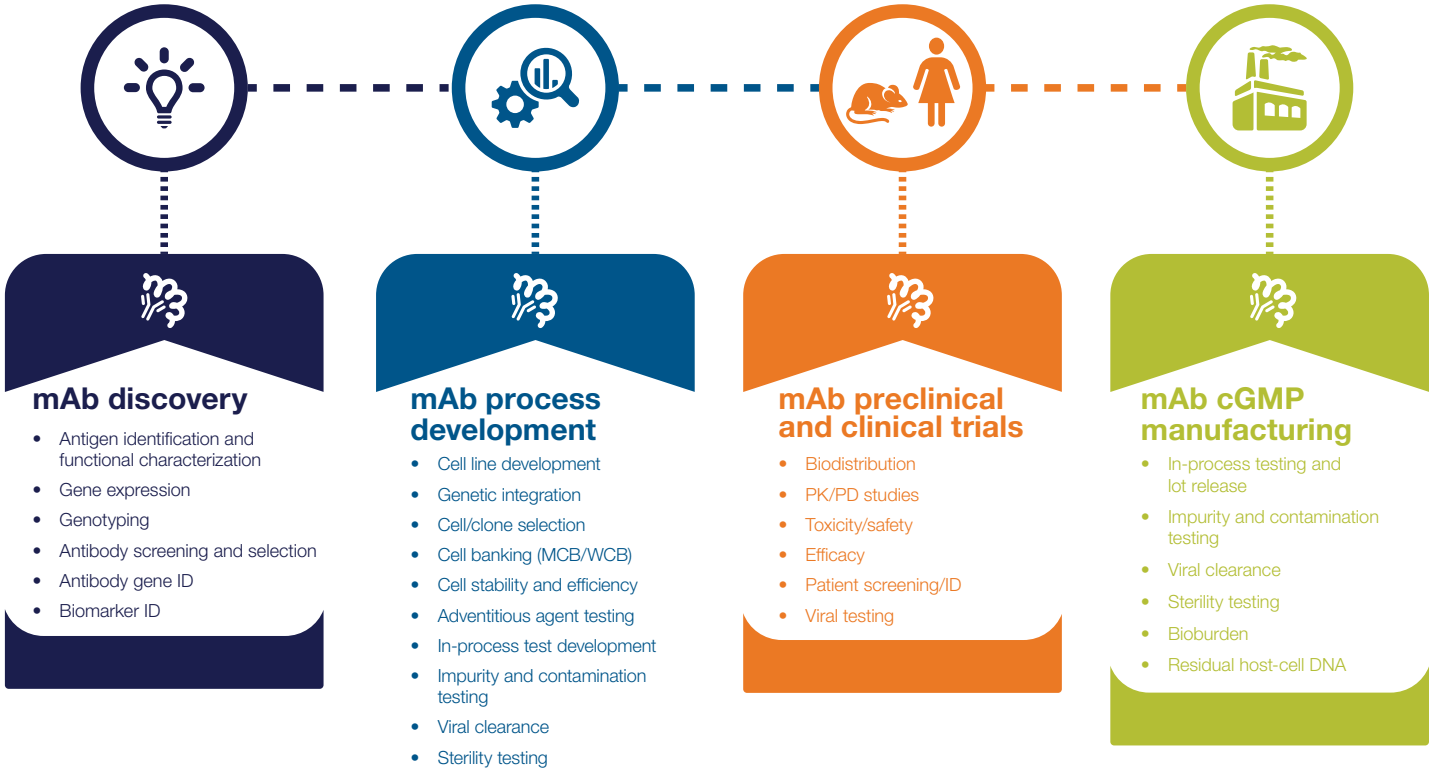
All **TaqPath qPCR assays and reagents** are manufactured in an FDA-registered and ISO 13485–certified facility in accordance with cGMP principles. Compliance documentation for all TaqPath assays and reagents includes statements of quality, site certifications, letters of stability, REACH compliance statements, supplier questionnaires, change notifications, and certificates of origin. All TaqPath assays are formulated to meet highly rigorous performance standards. Primer sequences and probes can be specified by the customer, and all formulations will be delivered in guaranteed yields. We perform extensive analytical QC and functional testing on all lots of **TaqPath master mixes** to help ensure the highest level of performance and lot-to-lot consistency for reproducible C_t values and wide dynamic ranges.

Use case: cell line development

Selecting a well-characterized clonally derived cell line for *in vitro* mAb production can help expedite the regulatory review process later on. To streamline development of stable and high-yielding cell lines, qPCR-based genotyping analysis can be performed to confirm at the genetic level that the pool of recombinant cells is stable and consistently productive after transfection with a mAb expression vector. Confirm proper transfection and insertion of the mAb gene by verifying the transgene sequence to help ensure clonally derived cell lines are pure before banking them. Doing this before process testing can save valuable time.

Use case: cell line banking

Periodic validation of transgenes in banked cell lines via genotyping analysis can enable highly sensitive, accurate, and comprehensive characterization of genetic identity and confirm continued transgene expression. Help ensure regulatory compliance by confirming the stability of cell lines for the master cell bank (MCB) and validate that the working cell bank (WCB) is genetically identical to the MCB.



To help ensure mAb production cell cultures are sterile, it is important to have a robust impurity and contamination QC testing program in place to monitor for DNA and RNA from viruses, bacteria, mycoplasmas, and other adventitious agents. Residual DNA and RNA from adventitious agents can cause serious adverse effects in patients, so it is important to use a trusted tool like qPCR for adventitious agent testing in the development phase. qPCR also enables a quantitative assessment of the effectiveness of residual nucleic acid removal procedures.

The **Applied Biosystems™ MycoSEQ™ Mycoplasma Detection Kit** and **ViralSEQ™ real-time PCR detection system** are ideal solutions for QC testing in regulated biopharmaceutical environments. Products like the **Applied Biosystems™ TaqPath™ BactoPure™ Microbial Detection Master Mix** and custom TaqPath assays provide ultimate flexibility for unique targets. The TaqPath BactoPure Microbial Detection Master Mix is functionally tested with an exhaustive panel of contaminant detection assays to confirm it is free of detectable extraneous DNA. A certificate of analysis (CoA) is available for each lot that includes a description of each QC parameter, method, acceptance criteria, and final conformity results, to deliver transparency for your downstream traceability requirements.

Use case: in-process test development and optimization

Real-time qPCR is useful for establishing sensitive and accurate genetic quality metrics to evaluate the safety, identity, strength, and efficacy of mAb production cells. These tests are also useful in the manufacturing phase. We recommend using our compliance resources to streamline processes. We can provide product usage statements and compliance documentation, including CoAs, certificates of origin, and ISO certificates by manufacturing site.

qPCR applications for preclinical and clinical trials

qPCR is a valuable tool for quickly identifying genes for relevant biomarkers and quantifying their expression during development and characterization of animal-based disease models for preclinical trials. Quantifying biomarker gene expression can also help mAb drug manufacturers refine their selection criteria for animal testing cohorts. With accurate, sensitive, and highly specific qPCR assays, manufacturers can assess the efficacy, purity, and safety of therapeutic mAb candidates in the preclinical stage.

Use case: subject screening and selection

Measure the expression of biomarker genes in target and off-target tissues to genetically characterize and classify animal-based disease models. Impurities and contaminants in mAb therapeutics are potentially detrimental, particularly those derived from host cells. Performing qPCR to screen individuals for viruses before testing is vital. By quantifying viral RNA and DNA with TaqPath custom assays, viral load in animal models can be monitored.

TaqPath custom assays are highly stable for reproducible and accurate quantitation of DNA and RNA in samples from various animal sources.

Use case: biodistribution studies

Since the concentration of a therapeutic mAb at its target site governs the magnitude of the pharmacological response, the processes that regulate distribution of the mAb in the body must be well understood.

The efficacy of a therapeutic mAb can be assessed by detecting and quantifying target gene expression in a suitable animal model with qPCR. This helps in determining whether the mAb is reaching its target or lying latent in off-target locations. The neutralization efficacy of an antiviral mAb can be assessed by detecting and accurately quantifying viral RNA or DNA with qPCR, and multiplex qPCR can enable manufacturers to identify different microbes and antibiotic resistance genes in a single assay.

Use case: pharmacokinetic (PK) and pharmacodynamic (PD) studies

PK and PD studies include tests for safety and efficacy, which are important because they reveal how drugs behave in the body and how the body responds to them. Understanding the behavior of a therapeutic mAb in relevant biological matrices like tissue and the possible influence of other proteins is necessary to understand its effects.

The exceptionally high specificity and accuracy of qPCR can enable identification of genes for disease-implicated proteins and receptors and quantitation of their expression. For example, mRNA half-life, distribution, degradation, and clearance can be monitored. qPCR is also a useful tool for evaluating the effects of changing the administration route of a drug, drug interactions, and combination therapy.

Use case: toxicology testing

The aim of toxicology studies should be to reveal any functional effects a therapeutic mAb has on major physiological systems. The expression of disease-implicated biomarkers, like miRNA, can be quantified with RT-qPCR to add genetic information to protein-based analyses of dosage and safety.

The antigenic specificity of a mAb, complement binding, and any reactivity or cytotoxicity in tissues that are not the intended therapeutic targets should also be understood in detail. qPCR is a valuable tool for quantifying target-cell DNA to assess tissue damage potentially caused by mAb administration to animals.

Manufacturing: the importance of qPCR for quality control

Performing qPCR analysis throughout the development and manufacturing of a therapeutic mAb to monitor changes in gene expression can help mitigate risk and accelerate production. It can also give manufacturers more confidence in their quality control systems.

We offer multiple tools to evaluate the quality of the manufactured drug substance and drug product at each step of the manufacturing process with product usage statements, certificates of analysis, certificates of origin, statements of quality, and change control and notification registrations for all TaqPath master mixes. ISO certificates by manufacturing site and material safety data sheets are available on request.

qPCR can be used to accurately assess the burden of microbial contamination in intermediates and final mAb products before sterilization to help satisfy regulated production environment requirements and maintain high safety standards. The same TaqPath master mixes and custom assays used to detect adventitious agents for clinical trials can be used to test for microbial DNA and RNA during mAb manufacturing. For DNA targets, use the **TaqPath BactoPure Microbial Detection Master Mix** with or without ROX dye. For RNA targets, use the **Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix** without ROX dye, **TaqPath™ 1-Step Multiplex Master Mix** with Mustang Purple™ dye, or **TaqPath™ 1-Step RT-qPCR Master Mix**.

Applied Biosystems™ qPCR instruments for mAb manufacturers

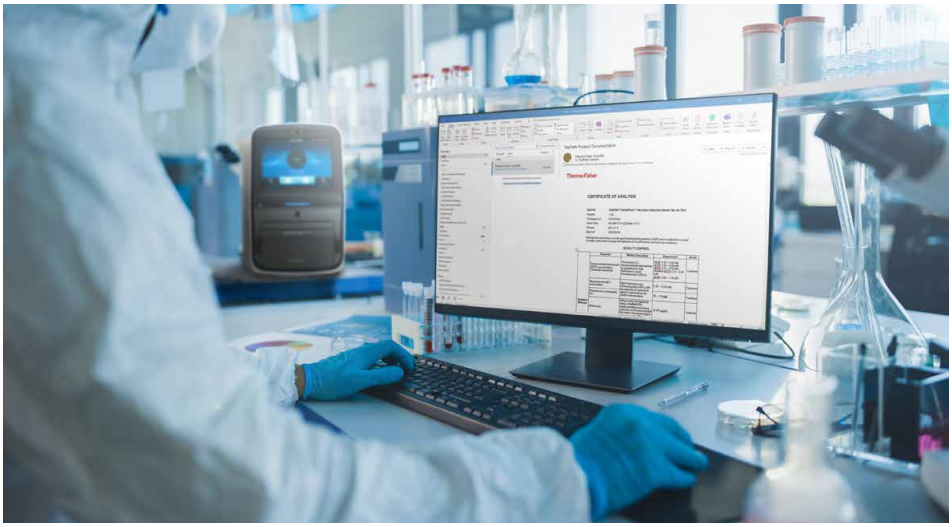
Effective and reliable **QualTrak real-time quantitative PCR (qPCR) workflows** for biopharma can help mAb manufacturers efficiently identify and characterize antigens, develop and validate clones and cell lines, and detect even low levels of contamination to help ensure the safety and efficacy of their products. Our suite of real-time qPCR instruments, qPCR assays, and qPCR reagents can help mAb manufacturers navigate the critical steps in each stage of development and production from discovery to manufacturing. Implementing QualTrak workflows can expedite time-to-market, and we can provide the services and documentation manufacturers need to maintain regulatory compliance.

The Applied Biosystems™ **QuantStudio™ 7 Pro Real-Time PCR System** is uniquely designed with innovative and time-saving features that help minimize user error and maximize uptime in the discovery phase. This real-time qPCR system is ideal for laboratories with multiple users and high-throughput requirements. The system enables hands-free operation through voice commands, remote operation, push-button help access, and automatic facial authentication. The **software for the QuantStudio 7 Pro Real-Time PCR System** is also compliant with 21 CFR Part 11 requirements for electronic records and signatures.

The QuantStudio 7 Pro Real-Time PCR System features:

- Hands-free operation with facial authentication at login, voice commands, and an RFID reader
- Remote access for managing experiments from outside the laboratory
- Automation compatibility for 24/7 operation
- A dynamic range that spans 10 orders of magnitude for detecting differences as small as 1.5-fold

We also offer a range of **instrument service plans** to help laboratories stay productive throughout the discovery phase of mAb development.



Absolute Q digital PCR assays

We offer our digital PCR tools when there is a need for absolute quantification for applications such as Applied Biosystems™ Absolute Q™ Viral Titer Digital PCR Assays, rare mutations, and target identification. Our dPCR assays enable easy, accurate, and absolute quantification of genomes. The assays can be run individually or multiplexed using a custom assay with your target gene of interest, to measure concentrations and evaluate quality for biopharma. The Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System is a plate-based digital PCR (dPCR) platform powered by proprietary **microfluidic array plate (MAP) technology** that enables all the necessary steps for dPCR—compartmentalizing, thermal cycling, and data acquisition—to be conducted on a single instrument. The dPCR workflow is identical to the qPCR workflow you are familiar with to improve ease of use, minimize hands-on steps, and maximize consistency.

QualTrak digital experience

Thermo Fisher can help expedite time-to-market for therapeutic mAbs with our complete QualTrak qPCR ecosystem. Save time and resources by visiting the **QualTrak digital experience**. This interactive tool is designed to help customers quickly find assays, instruments, cGMP-compliant reagents, compliance documentation, and qPCR workflows that fit their specific needs throughout mAb discovery, development, and manufacturing.

References

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