actome

# The PICO Handbook

## Guidebook for the Protein Interaction Coupling assay

Next Generation Discovery

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# What is PICO?

**Protein Interaction Coupling (PICO)** is an ultra sensitive immunoassay for the detection and precise quantification of **proteins, protein interactions,** and **post-translational modifications**. Our revolutionary approach combines the advantages of **immunoassays** with **digital PCR technology**, by translating protein status into DNA barcodes, and unlocks new dimensions in protein and interactomics research.

## With PICO you can...


... detect and quantify **proteins**, **protein interactions**, and **post-translational modifications**.

... employ **relative quantification** to compare biological samples with one another or perform **absolute quantification** to get precise molar amount of your target without the need of an external standard.

... analyze small samples volumes (~ 1 µL or just a couple of thousands of cells) with **femtomolar sensitivity** and **zero background**.

... measure up to two targets in parallel by **multiplexing**.

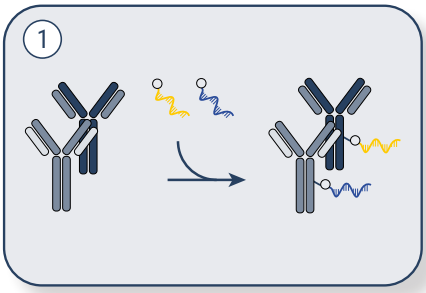
... prepare and analyze your protein targets using our **PICO kits** or take advantage of our **PICO Service** offers.

# How does PICO work in a nutshell?

The main innovation of PICO is the compartmentalized conversion of an antibody binding-based protein detection assay into a dPCR-based DNA detection event. The PICO workflow is based on a simple **label, mix, dilute, & detect** principle. This approach eliminates many potential pitfalls of classical immunoassays, such as western blot or ELISA, by offering ultra-high sensitivity, absolute quantification, zero background, and high parallelism potential.

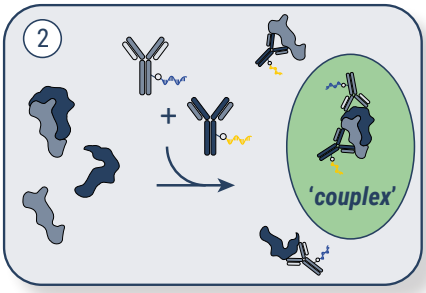
## The major steps in the PICO assay:

### 1. Antibody Labeling



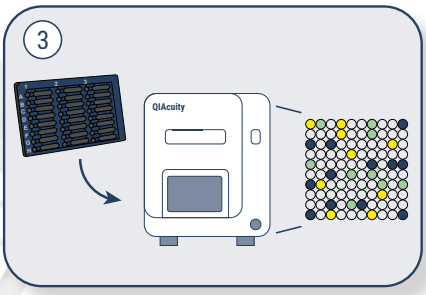

- Two different antibodies of your choice are labeled with unique DNA oligonucleotides (**PICO Labels**).
- The labeled antibodies are stable for at least **6 months** and the amount is sufficient for hundreds of PICO Assays.

### 2. PICO Assay



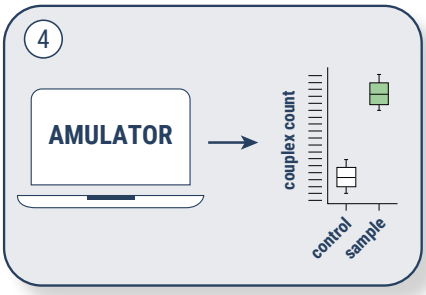

- The biological sample is lysed, mixed, and incubated with the labeled antibodies. '***Couplexes***', a target bound by the two different labeled antibodies, are formed.
- After incubation the sample is highly diluted and mixed with the matching **PICO Probes** and transferred to a dPCR assay plate.

### 3. dPCR Amplification




- In the dPCR assay plate the sample is **partitioned** into thousands of compartments.
- During **amplification** specific fluorescent signals are generated that are detected by the dPCR instrument.
- Compartments containing the couplexes have two fluorescent signals (e.g. yellow **AND** blue, thus depicted with green color).

### 4. Data Analysis

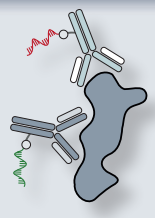



- The raw dPCR data is uploaded to **AMULATOR**, our web-based data analysis software.
- **Relative** quantification enables the relative comparison of target proteins between different biological samples
- **Absolute** quantification enables the precise determination of the target protein concentration in molar amounts.

# What can be a PICO target?

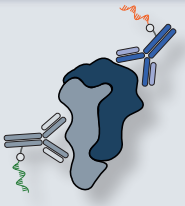
PICO can target **single proteins**, **protein interactions**, or **post-translational modifications**. For each target a pair of antibodies of your choice is required. A target bound by the two different antibodies, termed '***couplex***', is the molecular detection unit of the assay.

### Single proteins



- Use two antibodies binding to two different concurrently accessible epitopes
- Femtomolar sensitivity

### Protein interactions



- Use one antibody per interacting partner
- Optional cross-linking to preserve weak interactions

### Post-translational modifications


- Measure phosphorylations, acetylations, ubiquitylations etc.
- Gain insights into signaling pathways

## Two-color PICO assays

During the dPCR readout the fluorescent signals, generated during the amplification of the PICO Labels, are recorded. When performing a PICO assay with two colors (two different PICO Labels), you are able to analyze a **single target**. By precise selection of antibodies you are able to measure a variety of targets, which are depicted on the left.

For example if you are analyzing a **protein interaction**, the two different labeled antibodies will co-localize within the same compartment. Thus, this antibody pair produces a double positive signal that is counted. The same principle applies to the analysis of **single proteins** or **post-translational modifications** if both antibodies are directed against different epitopes on the same protein.

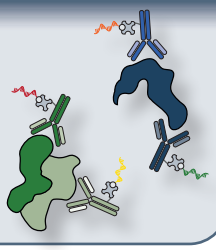
## Three- or four-color PICO assays

By performing three- or four-color PICO assays you can perform **multiplex PICO assays**. This allows you to detect and quantify two independent targets from the same sample simultaneously.

PICO also allows you to gain multiple information from a single target. For example you can measure the ratio of phosphorylation of a single protein (termed '**Triangular PICO assay**'). One antibody pair provides information on the total amount of protein while the other two antibody pair allow the detection of the phosphorylated version of the same protein.

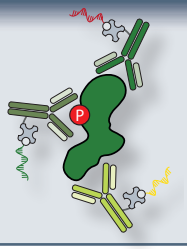
### Multiplex PICO assay

- Quantify two targets simultaneously from one PICO assay
- E.g. a single protein **AND** a protein interaction



### Triangular PICO assay

- Quantify the total amount of protein
- Quantify the amount of modified protein
- Calculate the **ratio** of e.g. phosphorylation



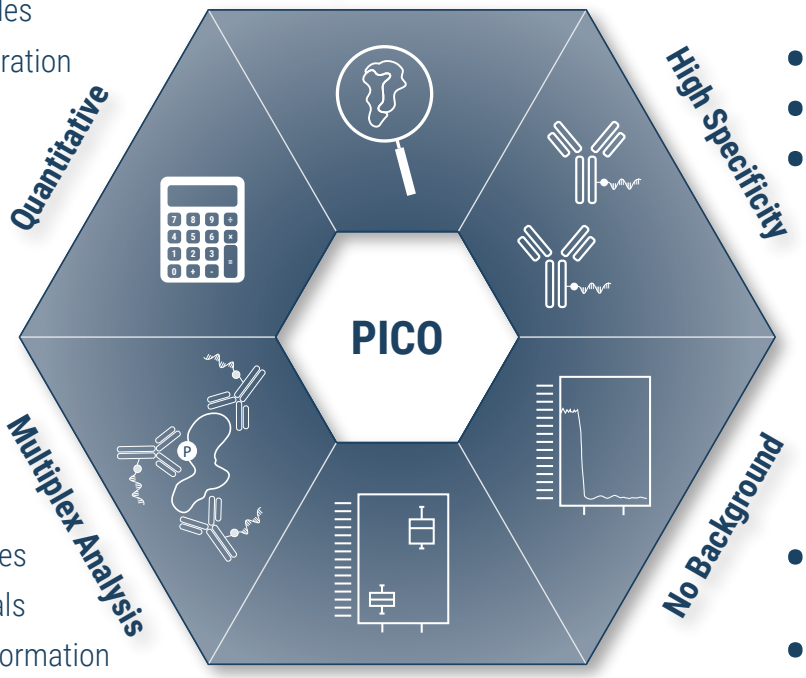
# What are the unique features of PICO?

- Femtomolar sensitivity
- Low sample volume
- Save time and money

**High Sensitivity**

- Relative - compare samples
- Absolute - molar concentration
- Molecular counting

**Quantitative**




**High Specificity**

- Two-antibody assay design
- No cross-reactions
- Reliable precise signal

**Multiplex Analysis**

- Up to 4 different antibodies
- Pairwise readout of signals
- One sample - multiple information

**No Background**

- Smallest quantities detectable
- Zero signal for negative samples

**Online Data Analysis**

- Fast graphical output
- Cloud-based workspace
- No extra costs

# What are the quantification options of PICO?

PICO enables both the relative and absolute quantification of target molecules using AMULATOR, Actome's on-line data analysis software. **Relative quantification** is best employed for direct comparison of biological samples while **absolute quantification** allows precise determination of the molar concentration of your target protein.

## Relative Quantification

- Single-target analysis: compare protein amounts between different biological samples
- Multi-target analysis: compare the amounts of different proteins in biological samples
- Calculate molar fold differences

## Absolute Quantification

- Measure precise molar amounts of your target molecule
- External reference is not required
- Measurement in a wide dynamic range

# The PICO workflow

Antibody Labeling

The first step in the PICO workflow involves the labeling of your chosen antibodies with unique DNA oligonucleotides (**PICO Labels**). The labeling is carried out by the **PICOglue Antibody Labeling Kit**, which is specifically designed for high efficiency (up to 100%) labeling reactions. Once labeled, the antibodies remain stable for at least six months, ensuring reliable and sensitive PICO assays. The antibody labeling process is designed as a three-day procedure with minimal hands-on time (<2 hours and 30 minutes) and generates sufficient amounts of labeled antibodies for hundreds of PICO assays.

PICOglue Antibody Labeling Kit

PICO Assay

The main part of the PICO assay is carried out using the **PICO Amplification Core Kit**. The kit protocol involves the lysis of the biological sample, the mixing of the lysed sample with the labeled antibodies, and the overnight incubation (termed 'binding reaction'). During the incubation the couplexes are formed. Next day, the sample is highly diluted and mixed with the matching **PICO Probes** and the dPCR Master Mix. The kit enables performing 120 PICO reactions in 24-well dPCR plates.

PICO Amplification Core Kit

dPCR

The dPCR partitioning and amplification is performed in **QIAGEN's QIAcuity dPCR System**. Each well, containing the sample, is partitioned into 26,000 individual compartments. Due to the high dilution, the partitioning leads to single target molecules in the compartments. The **PICO Labels**, bound by the matching **PICO Probes**, are amplified and specific fluorescent signals are generated. The QIAcuity dPCR System counts the amount of compartments with fluorescent signals and produces a binary output (signal vs. no signal).

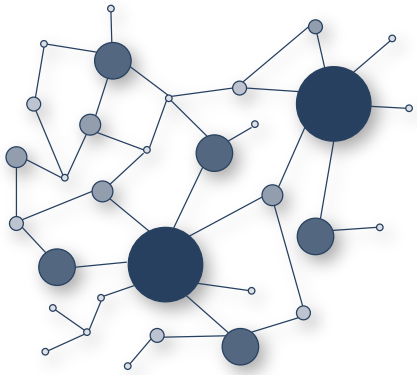
QIAGEN's QIAcuity One

Data Analysis

**AMULATOR**, our online data analysis software, processes the raw dPCR data and calculates the number of couplexes based on the fluorescent readings. The processed data is stored in a personal cloud-based workspace and remains accessible for further evaluation. AMULATOR also provides a graphical output of your data with a basic statistical evaluation. Data analysis with AMULATOR is included in the PICO kits without any additional costs.

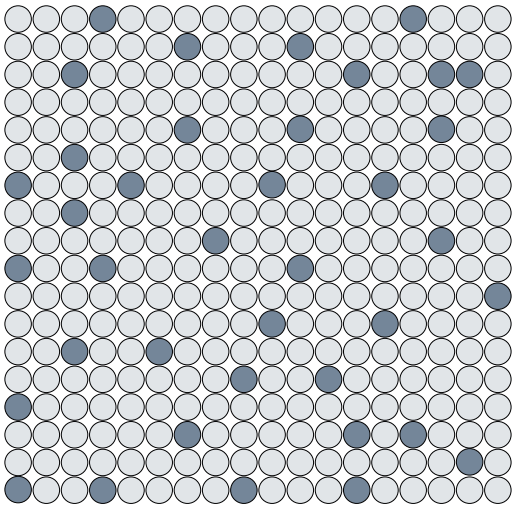
**AMULATOR**

## What is the interplay between proteomics and interactomics?



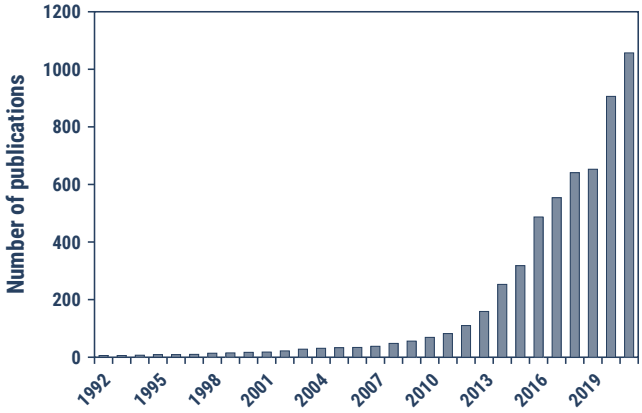
Each human cell contains around 19,000 to 21,000 protein coding genes that are translated into approximately 100,000 different proteins. At any given time and condition the total subset of the expressed proteins is called the **proteome**. Proteins are part of complex and post-translationally highly regulated protein interaction networks, also termed the **interactome**. The potential complexity of the interactome is estimated to be an order of magnitude larger than the complexity of the proteome itself. The proper functionality of the proteome and interactome and their regulation is crucial for cellular physiology driving scientific interest in their investigation. PICO is at the intersection of proteomics and interactomics research and offers a highly sensitive assay for detecting proteins, protein interactions, and post-translational modifications with a simple and productive workflow.

## What are the advantages of digital PCR?



The **digital PCR (dPCR) technology** is an extension of the classical PCR method that allows compartmentalized, single-molecular amplification and highly precise and sensitive quantification of DNA and RNA. The key extension of the dPCR technology is the partitioning of the sample into ten thousands of minuscule, nanoliter-sized individual compartments, each containing less than a single target molecule on average. The PCR reaction is carried out in each compartment individually and during amplification a fluorescent signal is generated that is detected by the dPCR instrument making the positive amplification reactions countable. The low volume of each compartment ensures amplification from a single DNA molecule and by applying **Poisson statistics** the original and absolute target DNA concentration can be very precisely calculated without an external calibration curve. Thus, dPCR enables **absolute quantification** of macromolecules.

**Number of publications using digital PCR throughout the years**




The dPCR concept was first described in 1992, however in recent years the dPCR technology has witnessed significant improvements, leading to an exponential surge in scientific publications. But does dPCR's potential end with DNA and RNA analysis? At Actome, we've pioneered PICO – a groundbreaking technology enabling the detection and quantification of **proteins, protein interactions,** and **post-translational modifications** using dPCR instruments.

## What does PICO measure exactly?

A standard PICO workflow employs two different labeled antibodies that are mixed and incubated with the lysed biological sample. During the incubation, termed 'binding reaction', both labeled antibodies bind to the same target, and '**couplexes**' are formed. The next day the binding reaction is highly diluted, mixed with the PICO Probes and dPCR Master Mix, and loaded onto a dPCR plate. Here's how couplexes are counted in the dPCR instrument:

- The dPCR instrument partitions the sample into thousands of compartments, creating individual compartments with different contents: unbound targets, labeled antibodies only, targets with only a single antibody bound, or couplexes.
- The PICO Labels hybridize with the matching PICO Probes and during dPCR amplification distinct fluorescent signals are generated: single color (e.g. yellow **OR** blue) for compartments with one PICO Label, and two colors (e.g. yellow **AND** blue, marked with green color) for compartments with two PICO Labels.
- A mathematical solution separates the real double positive couplex-containing compartments from the false double positive compartments, arising from the accidental co-compartmentalization of two different, unbound antibodies only.
- AMULATOR, Actome's web-based analysis software, counts the couplex-containing compartments and allows the quantification of your target protein concentration.

To sum up, PICO's innovative workflow and dPCR analysis offer a powerful solution to **precisely measure protein concentrations** through couplex formation. This allows researchers to gain insights into complex cellular processes with unparalleled accuracy and sensitivity.

## How sensitive is PICO?

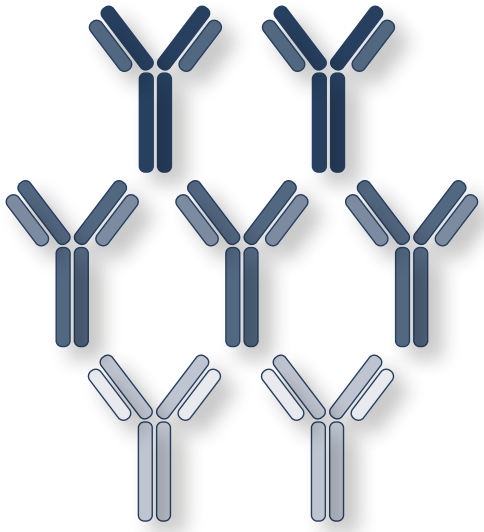
Since dPCR is used as the readout technique for PICO assays, all advantages of the dPCR technology such as **high precision, sensitivity, and absolute quantification**, are also implied for the detection and quantification of proteins by PICO. The LOD (limit of detection) is approximately 10 couplexes per dPCR reaction (one well in a 24-well QIAcuity Nanoplate). In addition, only a very **small input sample volume** (0.1 - 2 µL) is required ensuring high absolute sensitivity as well.

The targets are detected with **femtomolar sensitivity**, meaning PICO is able to detect approximately 600 - 60,000 molecules in 1 µL of sample depending on the affinities of the antibodies used. This calculates back to a total of 0.5 to 50 zeptomoles ($5 \times 10^{-20}$) of protein molecules.

This allows the user to generate reproducible and highly sensitive measurements even if the sample volume is very limited or proteins are of low abundance.

## What is the purpose of the high dilution prior to dPCR?

Due to the utmost sensitivity of the PICO assay, the binding reaction needs to be highly diluted to be measurable by dPCR. First, the number of couplexes per dPCR reaction needs to be between ten and a couple of thousands. Second, the amount of labeled antibodies per dPCR reaction, which is at best 5,000 (or a lambda of ~0.2[#]), is also of importance. The usual range of dilution to achieve this couplex concentration and lambda value is between 1 and 5,000 fold, depending on the affinities of the antibodies used. In special cases even no dilution is necessary. Importantly, the dilution affects the number of couplexes that can be detected, too high dilution can result in less than 10 couplexes per reaction, the LOD of the PICO assay. Our kit protocol provides a standard PICO setup that takes these factors into account and helps you run your initial PICO experiments.



[#] lambda is calculated by dividing the number of molecules by the number of partitions, in case of a 26k QIAcuity Nanoplate and the example above: 5,000/26,000 = ~0.2

## What are the antibody recommendations?

For the PICO assay a pair of antibodies is required for each target and the user is free to choose any antibody pair for their PICO assays. Below you can find our recommendations regarding the antibody selection process:

- The PICO assay works with both monoclonal and polyclonal antibodies. However, if possible, we recommend using monoclonal antibodies, since they are often better characterized and defined by the antibody producer and the batch-to-batch variabilities are minimal.
- The PICO sample preparation ensures the detection of proteins in their native, non-denatured conformation. However, there can be scenarios when the epitope is not available for antibody binding due to the native conformation of the protein burying the epitope or in case the protein interaction itself sterically hinders the antibody binding. Therefore, employing antibodies confirmed in non-denaturing applications such as immunofluorescence or flow cytometry might work better for PICO assays.
- Antibodies directed against a **protein tag** (e.g. **HA, FLAG, Myc, GFP,** etc.) can also be used and allows flexibility to detect and analyze tagged proteins.
- In general we recommend to choose antibodies with comparable $K_D$ values, as the antibody with a lower affinity limits the achievable sensitivity.
- If sensitivity is of importance for the particular PICO assay, we recommend choosing rabbit antibodies since in general they have higher affinities (lower $K_D$ values).

# Getting started with PICO Kits

Do you want to get acquainted with the PICO workflow and want to explore the ultra-high sensitivity of the PICO assay? For this purpose we offer the **PICO Protein Detection Trial Kit**. The kit contains a recombinant protein as a sample, two pre-labeled antibodies and all the reagents necessary to perform a PICO assay.

PICO Protein Detection Trial Kit

PICOglue Assay All-in-One Set

Or do you want to carry out the first PICO experiment with your own antibodies and sample? The **PICOglue Assay All-in-One Set** contains all the individual PICO Kits necessary to carry out the entire PICO workflow, from the labeling of antibodies to the PICO assay and dPCR reaction.

# Getting started with Actome's Services

In addition to the PICO kits, we also offer a range of services to help you utilize the PICO technology. Our dedicated specialists are ready to support you with services ranging from antibody labeling to the setup and optimization of custom PICO assays.

## PICO Antibody Labeling Service

With our PICO Antibody Labeling Service, your chosen antibodies are labeled with PICOglue Labels, delivering highly sensitive and specific probes for various PICO applications. Our specialists will assist you in choosing the most suitable antibodies tailored to your specific requirements.

## PICO Assay Development Service

Our PICO Assay Development Service offers a comprehensive solution for researchers, where we not only **develop** tailored PICO assays but also **optimize** them to ensure precise and reliable results for you. With Actome's expertise, you can focus on your research while having confidence in the assay's performance and accuracy.

## Custom Oligonucleotide Antibody Conjugation Service

The Custom Oligonucleotide Antibody Conjugation Service enables you to conjugate your antibodies with **custom oligonucleotides**, beneficial for various applications. Leverage our reliable and efficient PICOglue antibody labeling technology. We are happy to discuss your needs with you and provide best quality labeled antibodies for your projects.

# Ongoing public funded PICO projects

The EIC Accelerator **PICO-NGS** combines PICO and next-generation sequencing (NGS) applied to single cells and introduces a patented barcode strategy and innovative handling of single cells. Thus, enabling breakthrough analytical power, by measuring 50 targets simultaneously in single cells for the first time.

European Innovation Council

Co-funded by the European Union

The **nanodiagBW** future cluster brings together multidisciplinary research results that are on the edge of implementation and develops them into a generic platform that enables powerful diagnosis. By translating nanopore technology into innovative products and services, it contributes significantly to the earlier detection and more targeted treatment of common epigenetic diseases such as cancer, diabetes, cardiovascular, mental, neuro-degenerative, and infectious diseases.

## Notes

# Lets explore PICO together!

Watch our **PICO technology** video:

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