actome

What is PICO?

The Protein Interaction Coupling Technology!

Frequently Asked Questions

Next Generation Discovery

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General

What can be measured using the PICO assay?

With the PICO assay you can detect and measure single proteins, protein interactions and post-translational modifications. For a consistent nomenclature we call them 'target'.

What are the advantages of PICO compared to other protein detection assays?

The PICO assay has a high sensitivity (very low LOD) and in comparison to other techniques, no additional washing steps are performed that might reduce sensitivity. Furthermore, the assay has a large dynamic range allowing a robust detection of any target in a given sample with zero background.

What are the underlying molecular biological principles of PICO?

In a PICO assay, protein levels are translated into DNA levels by using differently oligonucleotide-labeled antibodies. A biological sample containing a target (target can be either a protein, a protein interaction or a post-translational modification) is incubated with two labeled antibodies. During the binding reaction the antibodies bind to their target(s) and form a complex. Two antibodies bound to one target are called 'couplex' and represent the detection unit of the PICO assay. After incubation and high dilution, the samples are compartmentalized and analyzed by digital PCR (dPCR). During dPCR, specific probes are binding to the labels, allowing the amplification and subsequently the detection of each label by a fluorescent signal. Compartments giving rise to both colors contain both antibodies. By comparing the results to a negative control, where the occurrence of a double positive compartment is explainable by applying Poisson statistics, Actome's AMULATOR software is able to calculate the amount of genuine couplexes.

What is a couplex?

Couplex is the molecular detection unit of the PICO assay. A couplex is defined as a target bound by two differently PICO DNA-labeled antibodies. A target can either be a single protein, a protein interaction or a post-translational modification.

Why are two antibodies necessary to detect a target?

If only a single antibody is used, distinction from target-bound and unbound antibodies in the dPCR reaction is not possible. However, two antibodies bound to a target and thus generating a double-positive signal in a dPCR partition can be distinguished from random co-localized double-positive partitions by the AMULATOR algorithm, therefore allowing the detection of couplexes.

What is a digital PCR and what are the advantages?

In comparison to a standard PCR or quantitative PCR (qPCR), during a digital PCR (dPCR) the sample is compartmentalized into thousands of partitions prior to amplification of the template. Fluorescent signals allow detection whether a partition contains a template. Here, the amplification of the template using primers and the generation of a fluorescent signal by using hydrolysis probes are combined. A high dilution of the sample in advance to partitioning assures that not all partitions contain templates and thus negative partitions, necessary for mathematical evaluation, are created. A major advantage of dPCR in comparison to other PCR methods is that the DNA can be quantified absolutely without a standard curve.

What are the underlying mathematical assumptions of dPCR?

Positive partitions in a dPCR (partitions containing amplified DNA template) are detected fluorescent signals. The distribution of templates in the partitions (the sample is compartmentalized into thousands of partitions in a dPCR) is following Poisson statistics. Knowing this, depending on the distribution of positive and negative

partitions, the amount of individual template molecules in the whole reaction can be calculated.

How long does a PICO assay take in the laboratory?

The assay takes two days. During the first day, the biological sample and the antibody mix are prepared and both are combined for the binding reaction, taking place over night. On the second day, the sample is highly diluted, dPCR Master Mix is prepared and combined with a dilution series of the sample and dPCR is performed.

What is the laboratory workflow of a PICO assay?

The first step is the preparation of the biological sample. The PICO assay can be performed using different biological sample types (any liquid sample like supernatant, blood but also cells), thus sample preparation may vary. In parallel, the specific antibody mix is prepared. The oligonucleotide-labeled antibodies need to be diluted, to ensure optimal sensitivity and precision of the assay. The biological sample and the antibody mix are then combined, allowing the binding reaction to take place. After overnight incubation, the samples are highly diluted to ensure optimal antibody concentration for the PICO assay. Then a dilution series of the sample is loaded onto QIAGEN's QIAcuity 26k 24-well Nanoplate and the dPCR is performed in a QIAcuity dPCR system. The data is analyzed and evaluated with Actome's AMULATOR software.

What is the Binding Reaction?

During the workflow of the PICO Amplification Core Kit the binding reaction takes place. Overnight incubation of the sample with the PICO DNA-labeled antibody mix allows the antibodies to bind to their target, establishing an equilibrium between the reaction partners.

How is the data retrieved from the QIAcuity and further analyzed?

The dPCR raw data (RFU values) can be downloaded from the QIAcuity software suite as csv-files (one for each channel used). Together with a sample definition file (excel sheet provided by Actome) they are packed in a zip-file and uploaded to Actome's AMULATOR software, a shared notebook on Observable. In order to upload your data, a login for your personal cloud-workspace (e.g. a Google account) and a token (received with the PICO Amplification Core Kit) are necessary. AMULATOR then calculates the number of couplexes from the raw data and a boxplot graph is created as direct graphical output with basic statistical evaluation. The data is stored and accessible for further analysis, can be shared with others and downloaded as csv-, png and svg-file.

What are the underlying mathematical assumptions of PICO?

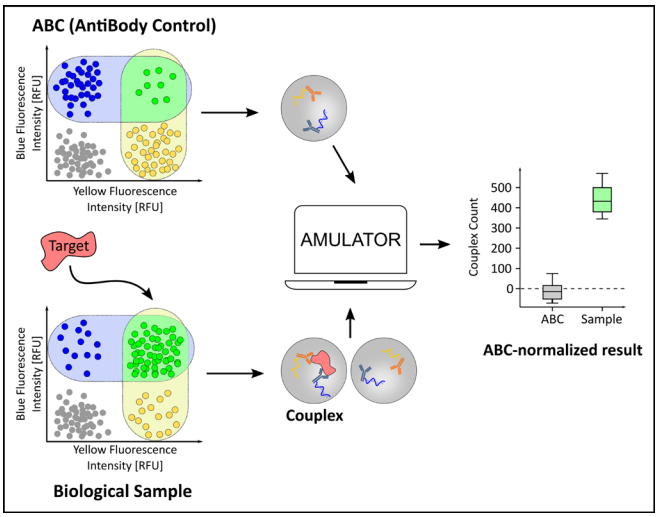

Figure: Depiction of couplex formation during PICO and couplex calculation with AMULATOR

One target bound by two PICO DNA labeled-antibodies is called couplex. Couplexes and unbound antibodies are subsequently detected in a dPCR. In parallel, an AntiBody Control sample (ABC) is measured. As in an ABC sample no target is present, the two PICO DNA labeled-antibodies distribute according to Poisson statistics among all partitions. Thus, the cluster of double positive partitions is the direct consequence of the overlapping antibody populations (upper left part of the figure). In contrast, the presence of a target will enrich partitions in the double positive cluster (lower left part of the figure) which is a deviation from Poisson distribution. Actome's algorithm can distinguish random and couplex double positive partitions to identify the absolute amount of real couplexes in the sample.

Which devices must be available in the laboratory to perform a PICO assay?

The PICO assay only requires QIAGEN's QIAcuity dPCR system and the QIAcuity dPCR consumables beside standard laboratory equipment like multi-channel pipettes, centrifuges or a ultrasonic bath. An optional step analyzing the antibody conjugation efficiency is given in the PICOact Antibody Conjugation protocol, which requires Agilent's Bioanalyzer or comparable devices.

Technical

What is lambda (λ) and how is it defined?

Lambda is defined as the average number of targets per partition. The average number of targets per partition (λ) depends on the sample concentration (C) and the number of partitions (n). Here, m is the number of targets in the sample and V_p is the partition volume.
$\lambda = m/n = C * V_p$

What is an ABC?

An ABC (AntiBody Control) is a control sample containing the antibody mixture (ABX) without the target (lysate). Hence, in a 2D scatter plot (fluorescence amplitudes of both antibodies) after dPCR, the double positive cluster can be explained by the Poisson statistical overlap of the two antibody populations. Ideally, after AMULATOR calculates the number of couplexes the ABC yields zero couplexes. However, due to dropout events and statistical offsets the ABC can differ from zero and thus the sample couplex count has to be normalized to the ABC couplex count.

What is a NTC?

A NTC (No Template Control) is sample free of template DNA and serves as a quality control to assure correct and careful laboratory practice. Digital PCR is a highly sensitive technology and during the PICO workflow reagents with high concentration of DNA are handled, so a NTC is necessary to confirm no accidental contamination has occured.

Why PICO does not need washing steps as other related assays, e.g. Proximity Ligation Assay (PLA)?

Actome's PICO labeling technology (carried out with the PICO aAC and aCALL Kit) guarantees a minimal free oligo concentration after antibody labeling. Therefore, every detected signal in the dPCR is expected to derive from a PICO DNA-labeled antibody. In addition, overnight incubation allows binding of the antibody to the target molecule at equilibrium concentration, allowing the maximum concentration of formed couplexes to be reached. Actome's AMULATOR software calculates the number of couplexes in the reaction.

What happens to unbound antibodies after binding reaction?

Unlike other assays such as Proximity Ligation Assay (PLA) or Proximity Extension Assay (PEA), unbound antibodies are not removed because there are no washing steps. On the contrary, unbound antibodies are pivotal for the couplex calculation as they determine the amount of random double positive partitions Additional double positive partitions can be explained by a target that is binding to both PICO DNA-labeled antibodies and thus enriching partitions in the double positive cluster.

What are the safety precautions if using PICO kits?

Please follow general safety precautions and wear protective gear (lab coat and gloves). Do not eat, drink or inhale any substances or apply them on skin/eye. Please rinse skin and eyes that came into contact with substances thoroughly. In case of exposure to any chemicals please seek immediate medical attention.

What is the shelf life of the purchased PICO reagents?

The PICO Antibody Purification Kit, the PICOact Conjugated Antibody Label Loading Kit, the PICO Amplification Core Kit as well as the PICOact Labels and PICO Probes can be stored up to 6 months when stored according to the kits manuals. The PICOact Antibody Conjugation Kit can be stored for up to 6 weeks when stored according to the kit manual. The expiry date can be found on the kits.

What additional materials and equipment are required for the PICO workflow?

Consumables

- 1.5 ml reaction tubes
- 0.5 ml low-protein binding tubes
- Pipettes and tips (1000 µl, 200 µl, 10 µl)
- Falcon tubes (50 ml, 15 ml)
- Syringes (5 & 20 ml, luer fitting)
- PCR microplate, 96 well, V- or U-bottom
- Sealing foil / adhesive film
- QIAcuity Nanoplate 26k 24-well

Devices

- Regular (1 - 1000 µl) & multichannel pipettes, 8-channel (10 - 100 µl & 30 - 300 µl)
- Vortex mixer
- Refrigerated Centrifuge & Table-top mini centrifuge
- Ultrasonic bath
- QIAGEN's QIAcuity Digital PCR System

Chemicals

- PBS (without calcium or magnesium)
- cOmplete ProteaseTM Inhibitor Cocktail (Roche)
- Anhydrous DMSO (for PICO aAC Kit)
- RNAse free water
- QIAcuity Probe PCR Kit

Antibody Conjugation

How long does the conjugation process take?

The workflow of the PICOact Antibody Conjugation Kit is designed as a two-day procedure with a hands-on time of approximately 40 minutes. On the second day, the labeling process of the antibody with the PICOact Conjugated Antibody Label Loading Kit can be finished.

What are the requirements/recommendations concerning the used antibodies?

The PICO assay works with every antibody, however, if possible we recommend using monoclonal antibodies. Also the antibody with highest possible binding affinity (KD) should be used; in general antibodies produced by the animal host rabbit show higher affinities compared to others. If the antibodies are stored in a buffer containing BSA, primary amines or other chemicals (e.g. azide, TRIS) that can interfere with the labeling reaction, we recommend purifying the antibody with our PICO Antibody Purification Kit prior to the labeling process.

How long can Actomidin-conjugated and PICO DNA-labeled antibodies be stored?

Both Actomidin-conjugated as well as subsequently PICO DNA-labeled antibodies can be stored for at least two months at 4°C. However, we recommend using the PICO DNA-labeled antibodies as fast as possible. In

addition, we recommend comparing only samples prepared in the same experiment with each other.

How is the conjugation efficiency determined?

The PICOact Antibody Conjugation Kit enables conjugation of antibodies with Actomidin with an efficiency of up to 90%. However, to determine the exact conjugation efficiency, an optional protocol is given in the PICO aAC protocol using Agilent's Bioanalyzer where the relevant peaks of the gained electropherogram of a conjugated antibody in comparison to an unconjugated antibody are compared. See the PICO aAC manual for a detailed protocol.

Does the conjugation efficiency have to be determined?

Due to the high performance rate of the PICO aAC Kit, determination of conjugation efficiency is not necessary, however, we recommend it as an additional quality control step if possible, especially if you are labeling antibodies for the first time.

What is an acceptable conjugation efficiency for the PICO assay?

The PICO aAC Kit is characterized by a high performance rate and under right experimental conditions, conjugation efficiency of up to 90% can be achieved. For the PICO assay, we recommend a minimal conjugation efficiency of 50%.

Antibody Labeling

How long does the labeling process take?

The workflow of the PICOact Conjugated Antibody Label Loading Kit is designed as a one-day procedure with a hands-on time of approx. 50 minutes.

How stable are the PICO DNA-labeled antibodies?

Both the Actomidin-conjugated as well as the subsequently PICO DNA-labeled antibodies are stable for at least six months at 4°C. However, we recommend using the PICO DNA-labeled antibodies as fast as possible.

How is the concentration of PICO DNA-labeled antibodies determined?

The concentration of labeled antibodies is determined via digital PCR using the QIAcuity dPCR system and components of the PICO Amplification Core Kit as well as the PICO Probes. A serial dilution of the antibodies are analyzed according to the PICOact Conjugated Antibody Label Loading manual.

What is an acceptable concentration for a PICO DNA-labeled antibody?

In order to perform a PICO assay we recommend that the concentration of the labeled antibody should not be below $1x10^9$ cp/µl. In general, the higher the concentration of the labeled antibody, the more PICO reactions can be performed.

What is the last-wash control of the PICO DNA-labeled antibody and what is an acceptable result?

The last-wash control is gained during the last wash step when an antibody is labeled with the PICOact Conjugated Antibody Label Loading Kit. During the labeling procedure unbound DNA-labels are removed by several washing steps. Comparing the flowthrough of the last washing step to the labeled antibody by dPCR allows the calculation of the percentage of unbound labels in the final labeled antibody stock. For this, the ratio of the PICO DNA-labeled antibody concentration and the label concentration in the last-wash control is calculated. For the PICO assay, a free label content below 3% is recommended.

Biological Material

What kind of samples can be used in a PICO assay?

In general, every liquid sample can be analyzed with the PICO assay, e.g. supernatant, blood serum, etc. The only requirement is that the samples must be liquefied, lysed, and homogenized, following the general laboratory instructions for a particular sample. In case of measuring cells (human cell lines, bacteria, etc.) a lysis step is included in the PICO Amplification Core Kit manual.

What amount of cells are required for PICO?

In general, we recommend a concentration of 10,000 cells/µl and a total number of 1 mio cells because they are easier to handle. Since the abundance of proteins can vary in a broad range, a minimal amount of cells is hard to determine. However, due to the high sensitivity of the PICO technology, a lower number of cells will also work when the protein is expressed in sufficient amounts.

Can cryopreserved cells be used for PICO?

Yes, cryopreserved cells can be used for PICO assay. A reconstitution period of 10 min in full cell culture media after thawing is recommended.

How long can lysed cells be stored?

We recommend using lysed cells directly, however, the lysate can be stored at 4°C for up to 4 days.

AMULATOR

Do I need any additional software and how can I access AMULATOR?

Other than the AMULATOR no additional software is required to analyze the raw dPCR data that can be downloaded from the QIAcuity software suite. AMULATOR is offered as a web-based application. A public Observable notebook is provided, where a personal user account (e.g. a Google account) is required to access the personal data storage space. Here, the data of previous experiments can be reviewed, new data can be uploaded and analyzed. To upload a new dataset a token is needed, which is provided with each PICO Amplification Core (AMC) Kit.

How is the data displayed and is it customizable?

The number of couplexes in each dilution series is calculated from the raw dPCR data by Actome's AMULATOR software. Based on the technical replicates, a boxplot graph with basic statistical evaluation, is created for direct visualization of the experimental results. The boxplot graph can be downloaded as a raster-based image (png-file) or a vector-based file (svg-file) for further personalization. The results are also available as a spreadsheet (csv-file).

Where is the data stored and is it safe?

The personal data of each user is saved on our cloud-based workspace and remains accessible for further analysis or re-evaluation. Actome respects and protects the personal and proprietary data of each customer and ensures highest standards of data protection (see also terms of service and data policy for details).

For further information visit www.actome.de and consult The PICO Handbook!

For any questions or inquiries don't hesitate to contact us at info@actome.de