EV Q Kit
USER MANUAL

Content

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PICO BioScience GmbH

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01 Kit Contents and Storage

EV Q Kit		
Catalog Number:		PICO-0000140
Pouch - store at 4°C	**pcs**	**Item code**
Bovine serum albumin	5	PICO-000010 C
Pouch - store at -20°C		
Coupling dPCR Mix	1	PICO-000010 E

The PICO EV Q Kit ships at room temperature. Upon arrival, immediately store components at their optimal temperatures (noted on pouches). Note the different required storage temperatures. Expiration dates are on the back of the pouches.

02 Intended Use

The PICO EV Q Kit is for research use only (RUO) and not for diagnosis, prevention, or treatment of disease. Manage the product with care and adhere to national safety guidelines.

03 Safety Information

Always wear a lab coat, disposable gloves, and protective goggles when handling chemicals. Consult the appropriate material safety data sheets (MSDS) for more information. These are available at: www.actome.de/resources/safety-data-sheets

For reagent spillage, absorb, dispose of, and clean with laboratory detergent and water. If the spilled liquid is potentially infectious, clean first with detergent and water, then with 1% (w/v) sodium hypochlorite.

04 Quality Control

Each PICO EV Q Kit is evaluated against predetermined specifications to ensure consistent product quality.

05 Introduction

Protein Interaction Coupling (PICO) is an immunoassay utilizing the QIAcuity Digital PCR System to characterize and quantify intact extracellular vesicles (EVs). The EV Q Kit supports both 24- and 96-well QIAcuity Nanoplates (26k and 8.5k), allowing for 150 or 600 PICO reactions (five plates each). Appropriate PICO BL, P8, N6, or O7 Probes are necessary based on the PICO Labels used. The PICO EV Q protocol is a two-day procedure with minimal hands-on time (< 1 h 30 min) (Figure 1).

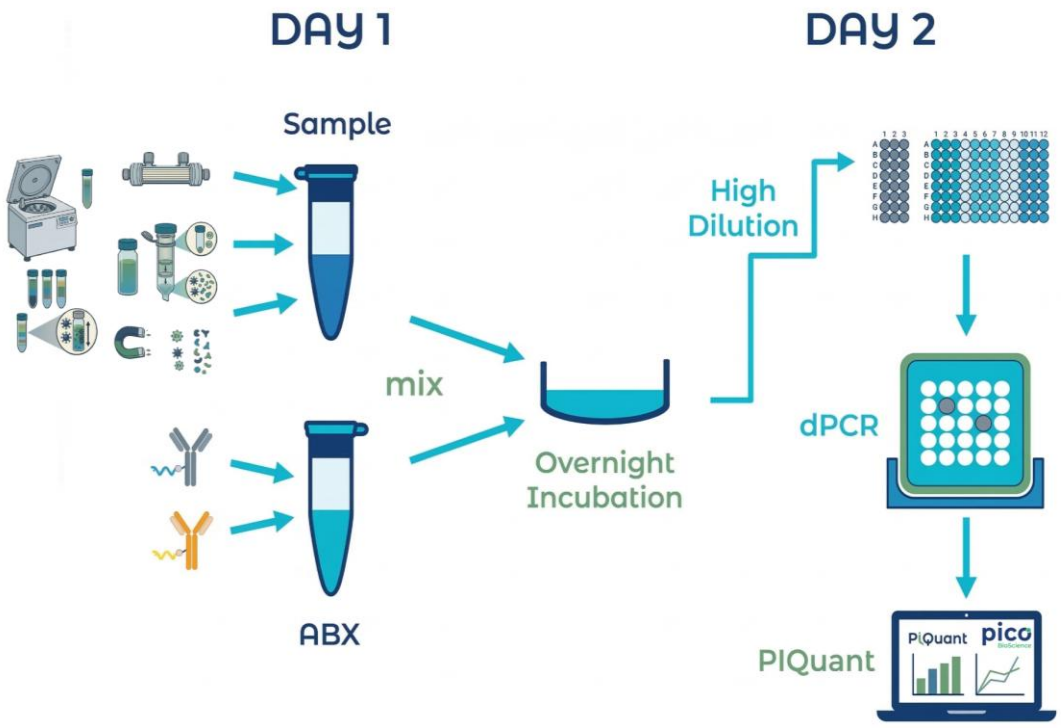

Figure 1 PICO EV Q Kit Workflow

The EV Q Kit uses two, three, or four different labeled antibodies to detect and quantify extracellular vesicle (EV) surface proteins. For single-protein EV detection, one monoclonal antibody is split and labeled with two different PICOglue Labels. Multiple EV surface proteins require one antibody per marker. PICO quantification also allows for precise counting of intact EVs and their sub-populations. Learn more in our application note and order pre-labeled antibodies (e.g., CD9, CD63, CD81) for EV analysis via our EV-LABs.

06 Preparation for a PICO Assay

06.1 General Remarks

- The PICO assay requires precise pipetting. Always use calibrated pipettes for accurate results, especially at high dilution factors. Mix gently by pipetting up and down to avoid spills and material loss.
- Change gloves frequently to prevent contamination, which can be detected if the NTC (no-template control) shows positive signals.
- For best results, use four technical replicates per sample. Include both NTC and antibody control (ABC). The ABC contains only antibodies (no sample) and should ideally show zero couplex counts. Any deviation is automatically corrected by the PIQuant software.
- The Lambda value should be below 0.55, with 0.15 being optimal. A lower Lambda improves couplex-detection accuracy by reducing biases, but setting it too low decreases assay sensitivity and limits the detection of statistically significant couplexes.
- Note: Sensitivity depends on partition count. Using the 8.5k 96-well plate reduces sensitivity by ~3x compared to the 26k 24-well plate.

06.2 Concentration of Antibodies in the Binding Reaction

PICO assays require at least two antibodies per target, which should bind to two different, concurrently accessible epitopes.

- **For relative quantification:** Use **40 pM** antibody in the binding reaction.
- **For absolute quantification:** Use **500 pM** (saturated conditions).

06.3 Considerations Regarding Biological Material

Biological Material		
Feature	Main criteria	Additional criteria
Sample type	Isolated EV samples	Choose your preferred method for EV isolation
Sample preparation	EV Sample dependent	● Intact EVs suspended in PICO BioScience's EV buffer (EVB) to quantify EVs based on surface markers

- For EV samples, we recommend performing a Dilution Curve (DC) to titrate the optimal EV concentration that allows robust detection while saving biological material. For instance, a 5-fold dilution series with five dilution steps can be prepared in EVB. This results in a dilution series of undiluted, 5-, 25-, 125- and 625-fold diluted samples.

06.4 Exemplary Setup of a Binding Reaction for a 24-well dPCR Nanoplate

Plate layout of an example binding reaction for a 24-well PICO experiment. In this example, five samples, with four technical replicates each, were used. For the ABC, three technical replicates are recommended.

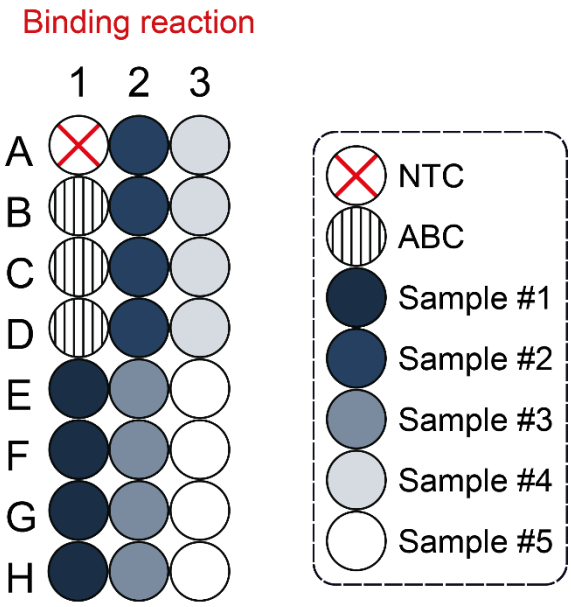

Figure 2 Example Binding Reaction Setup

Notes

07 Equipment and Reagents to be Supplied by User

Note: Equipment and reagents required to prepare biological material are not listed below and depend on the input material.

Devices/Equipment

- QIAcuity Digital PCR System (QIAGEN, Cat. #: 911001)
- Table-top mini centrifuge for quick spins (~1,000 rcf)
- Plate centrifuge (e.g., Megafuge 8 (Thermo Fisher, Cat. #: 75007210)
- Vortex mixer
- Multichannel pipette, 8-channel (1-10 µl)
- Multichannel pipette, 8-channel (10 – 100 µl)
- Multichannel pipette, 8-channel (30 – 300 µl) (optional)
- Regular 1-channel pipettes (1 – 1,000 µl)
- Electronic Multichannel pipette (INTEGRA, Cat. #: 4722), 12-channel, 5-12.5 µl (optional)

Consumables

- QIAcuity Nanoplate 26k 24-well (QIAGEN, Cat. #: 250001) or QIAcuity Nanoplate 8.5k 96-well (QIAGEN, Cat. #: 250021)
- PCR microplates, 96-well, Polypropylene, V-bottom or similar
- Sealing Foil Adhesive Film (e.g., Thermo Fisher, Cat. #: 10696771)
- 12.5 µl GRIPTIP, Sterile, Filter 5 Racks of 384 Tips, long; for electronic multichannel pipette (INTEGRA, Cat. #: 3405) (optional)
- 1.5 ml reaction tubes
- 0.5 ml reaction tubes
- 0.5 ml low protein binding tubes (e.g., Eppendorf, Cat. #: 0030108094)
- 10 µl, 200 µl, 1,000 µl standard pipette tips
- 15 ml Falcon tubes

Chemicals and Kits

- QIAcuity Probe PCR Kit (QIAGEN, Cat. #: 250101) (~ 1.5 ml)
- PICO Probes (PICO P8, BL, N6, O7 Probe, Cat. #: PICO-000070-73)
- Phosphate-Buffered Solution (PBS), without calcium or magnesium ions (e.g., Thermo Fisher Scientific, Cat. #: 12037539)
- cOmplete Protease™ Inhibitor Cocktail (Roche, Cat. #: 04693132001)
- Ultrapure water (e.g., Thermo Fisher Scientific, Cat. #: 15667708)

08 Protocol of PICO EV Q Kit

Note: This PICO protocol, for intact EV detection using up to four labeled antibodies, details setup for both QIAcuity Nanoplate 26k (24-well) and 8.5k (96-well) plates. Note: The 8.5k plate has fewer partitions, reducing sensitivity.

08.1 Buffer Preparation for intact EV characterization based on surface markers

1. Prepare the chemicals and buffers as listed below. The volumes can be adapted to the needs (e.g., if required, more EV Buffer can be prepared for the dilution series).
   The buffers can be kept for specified periods of time and used for further experiments. Prepare the buffers directly when they are used.

BSA (5x stock)
Add 400 µl PBS
Stable for 3 days at 4°C

EV Buffer (EVB)
100 µl BSA
400 µl PBS
Prepare the Control Buffer fresh

PICOglue Storage Buffer
20 µl 10x PICOglue Antibody Storage Buffer
180 µl PBS
Stable for 6 months at 4°C

08.2 EV Isolation and Storage

Use any preferred EV isolation method or commercial kit. If a commercial kit is used, the isolated EVs must be re-buffered before the binding reaction, though they may remain in the isolation elution buffer beforehand.
Immediate analysis of the isolated EVs is recommended. For short-term storage, keep the EVs at 4°C for a maximum of 4 days. For long-term preservation, aliquot the EVs and freeze them at -80°C.

08.3 Binding Reaction

2. Calculate the volume of antibody stocks and EVB required for the antibody mix (ABX) using the PICO Calculator (Step 2 in the 'PICO Calculator' tab).

Antibody dilutions for binding reactions, if prepared in 1x Storage Buffer, can be stored up to 12 months at 4°C. Highly diluted stocks of 200-500 times, may be unstable; an intermediate dilution of 20-200 times in 1x Storage Buffer is recommended for storage.

3. Prepare the ABX using EVB. Then add the calculated volume of the antibody stock solutions or antibody pre-dilutions.

   Prepare a minimum of 60 µl of ABX for a QIAcuity Nanoplate 26k 24-well and 210 µl for a QIAcuity Nanoplate 8.5k 96-well. Recommended antibody working concentrations are 40 pM for relative quantification and 500 pM for absolute quantification (selectable in the PICO Calculator).

   Example for 4 antibodies with a concentration of 1.93E^{10} cp/µl:

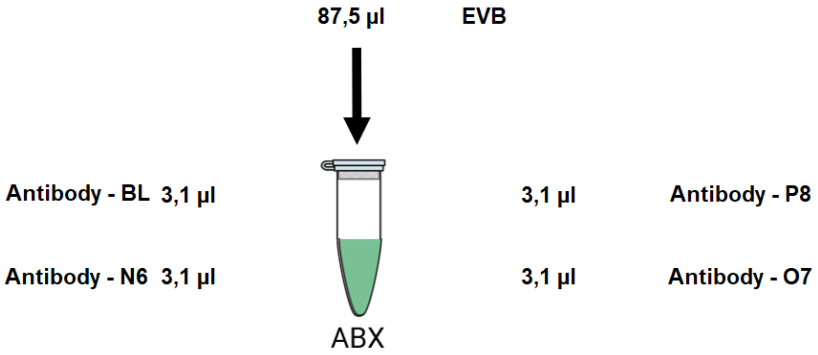

Figure 3 Example ABX pipetting scheme for 4 antibodies

4. After preparation of the ABX, the EV samples prepared in *section 08.2* can be diluted or used directly for the following binding reaction.

5. Set up the binding reaction in a 96-well PCR microplate (v-bottom) (see example in **Section 06.4**). Add 4 µl of EVB to a dedicated well for the NTC.

6. Combine 2 µl of EVB with 2 µl of ABX and make at least three technical replicates for the ABC control

   Due to the small volume of the binding reaction, do not mix by pipetting. The volume of lysate and ABX can be adapted. This must be considered in the calculations of the ABX and the pre-dilution. Please keep in mind that multiple different ABC control samples might be necessary if different antibody mixes are used in one experimental setup.

7. Combine 2 µl of the EV sample with 2 µl of ABX. Good laboratory practice recommends using at least four replicates.

8. Seal the plate (without the lid) with an adhesive foil. Vortex the plate for thorough mixing. Centrifuge the plate to collect the liquid at the bottom (~1,000 rcf, 30 s) and incubate at 4°C overnight.

   The incubation time can be varied between 12-24 hours.

08.4 Pre-dilution and Digital PCR

9. Prepare the Master Mix for 24 or 96 samples, ensuring it contains the required PICO Probe(s). If fewer than four labeled antibodies are used, substitute the PICO Probe volume with ultrapure water (see amounts below). Vortex for 10 s and spin down (~1,000 rcf, 5 s). The Master Mix can be prepared up to three days in advance.

Master Mix		
Reagents	**24-well plate**	**96-well plate**
Ultrapure water	606 µl	834 µl
QIAcuity Probe Master Mix	284 µl	390 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
Coupling dPCR Mix	36 µl	50 µl

Note: If you use less than 4 Probes, add the respective amount of ultrapure water to the Master Mix.

10. PICO Assay Preparation Instructions

For a 24-well PICO Assay:

Figure 4 Dilution scheme for 24 well Nanoplates

a. Prepare a new 96-well plate for the dilution steps.
b. Add 120 µl of PBS for a 500 pM ABX concentration or 33 µl of PBS for a 40 pM ABX concentration to columns 1-3 (for Dilution Series 1, DS1) and columns 5-7 (for DS2).
c. Add 41 µl of Master Mix to columns 10-12.

For a 96-well PICO Assay:

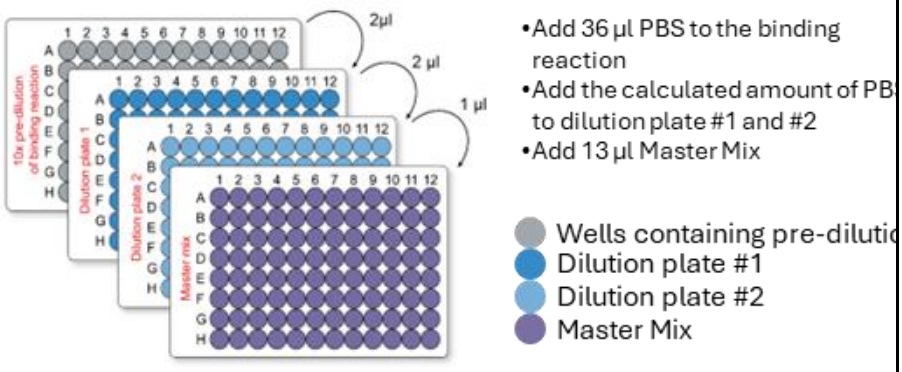

Figure 5 Dilution scheme for 96 well Nanoplates

a. Prepare three 96-well plates: Dilution Plate #1, Dilution Plate #2 filled with 143 µl PBS when using a 500 pM ABX concentration or 39 µl PBS for an ABX concentration of 40 pM.
b. Master Mix Plate #3 is filled with 13 µL of Master Mix.

11. Remove the adhesive foil from the incubated sample plate. Add 36 µl of PBS to the 96-well plate containing the binding reactions (represents the recommended first 10x pre-dilution). Mix thoroughly by pipetting up and down.

    Perform the following steps without unnecessary breaks, as the dilution disrupts the equilibrium binding conditions and induces antibody dissociation.

12. Transfer 2 µl volume from each pre-diluted sample into the corresponding wells of the dilution plate (DS1/Dilution plate #1). Mix by pipetting up and down 10 times.

13. When performing the second dilution step, transfer 2 µl volume from the first dilution step into the corresponding wells of the dilution plate (DS2/Dilution plate #2). Mix thoroughly by pipetting up and down.

14. Finally, transfer **1 µl** from the last dilution step into the wells containing the Master Mix. Mix thoroughly by pipetting up and down.

15. Transfer 40 µl (24-well) or 12 µl (96-well) of the diluted sample Master Mix to the QIAcuity Nanoplate. Seal and insert the Nanoplate into the QIAcuity dPCR System. Run the dPCR program using the following dPCR settings:

Priming			
QIAGEN Standard Priming Profile			
PCR conditions			
Step	**Temperature**	**Time**	
Hot-start	95°C	2 min	
Denaturing	95°C	15 s	Cycle 40 times
Annealing	58°C	30 s	

Imaging conditions			
PICO Probe	**QIAcuity channel**	**Integration time**	**Gain**
P8 Probe	FAM, green channel	500 ms	6
BL Probe	HEX, yellow channel	400 ms	6
N6 Probe	TAMRA, orange channel	400 ms	6
O7 Probe	ROX, red channel	300 ms	4

09 Evaluation

For the Evaluation with the PIQuant Software please use this link. To get more information, consult the PIQuant User Manual or contact our support team here.

10 Troubleshooting Guide

If you encounter any issues, please refer to the troubleshooting guide provided below. Additional support is available here.

Troubleshooting	
Issue	Comments and Suggestions
Lambda value in PICO assay not in range (0.01-0.6)	
Antibody concentration determined during quality control of labeled antibodies was not correct	Recalculate antibody concentrations using the data of the PICO assay and repeat the assay with the new concentrations. For this, the antibody concentration of each antibody found in the 'Current Results' file of the PICO assay is multiplied with the dilution factor back to the antibody stock for the corresponding antibody (which can be found at the bottom of the PICO Calculator).
No couplexes or small numbers of couplexes detected	
Antibody concentration determined during quality control of label loaded antibodies was not correct	Check if lambda value is in range (0.01-0.6), if not recalculate concentrations based on the data of the PICO assay and repeat the assay with the new concentrations.
Wrong default threshold of fluorescence intensity (RFU) was set in the QIAcuity software suite	Select '1D Scatter Plot' in analysis mode of QIAcuity software suite and adapt the thresholds.
Sample concentration outside the dynamic range of the PICO assay	Perform a longer dilution curve to find the optimal concentration of target detection. Too high target concentration can result in lack of couplex detection.

11 Ordering Information

PICO kits can be purchased directly from shop.actome.de or a quote can be requested from sales@actome.de. Supporting materials are available on https://landing.actome.de/resources or can be requested from our Customer Support (support@actome.de).

Ordering		
Product	Description	Cat. #
PICO EV Q Kit	dPCR detection for PICO assays for intact EV detection and quantification (5 x 24 reactions; 5 x 96 reactions)	PICO-000140
PICO Probes	P8 (FAM), BL (HEX), N6 (TAMRA), O7 (ROX) for detection in dPCR (5 x 24 reactions; 5 x 96 reactions)	PICO-000070 - 73