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iPCR Conversion Kit

Catalog #: NB-iPCR42 (96 wells)

User Manual

This kit is designed to convert an ELISA to highly sensitive immune PCR.

Manufactured and Distributed by:

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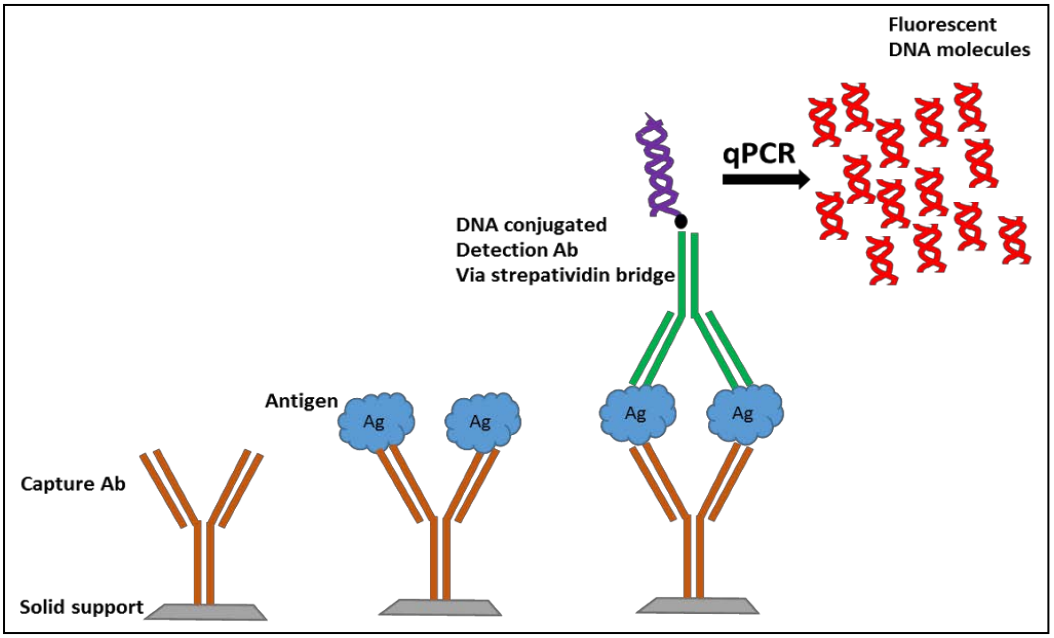
Description

Immuno-PCR (iPCR) is a powerful method for detecting ultra-low quantities of antigens. It combines the advantages of both enzyme-linked immunosorbent assay (ELISA) and PCR in specificity, sensitivity and easy to adapt. Despite its potential, iPCR is an underutilized method as evidenced by the low number of publications on its routine application and unavailability of validated, ready-to-use commercial kits. To make it possible for the researchers to detect various low-abundant analytes, Novatein Biosciences developed validated and ready-to-use iPCR conversion kit and iPCR kits.

Assay principle



Materials Supplied

Components	Amount
Biotinylated DNA (100X)	1 vial
Streptavidin (100X)	1 vial
Primer mix (with taqman probe) 100X	1 vial
PCR mastermix (2X)	1 vial
Wash buffer (25X)	25 ml
Blocking Buffer (10X)	12 ml
Buffer B (10X)	25 ml

Materials required but not supplied

- ☐ Capture and biotinylated detection antibody.
- ☐ Antigen standards.
- ☐ Top yield plates or high binding ELISA plates

Reagent Preparation

Dilute capture antibody in PBS (or as recommended by the manufacturer)

Dilute biotinylated detection antibody in blocking buffer

Dilute streptavidin 1:100 with blocking buffer

Dilute biotinylated DNA 1:100 with nuclease free water

Dilute 25X wash buffer to 1X with water

Prepare 1X PCR mix by diluting PCR master mix, primers to 1X with water.

Assay Procedure

1. Dilute the capture antibody (0.2 – 5 µg/ml) in PBS and add 30 µl to each Topyield plate well. Seal the plates with the adhesive cover and incubate overnight at 4°C (or as recommended by the manufacturer).
2. Wash 3X with wash buffer (300 to 330 uL/well), each time incubating for 1 min at room temperature.
3. Add 300 µl blocking buffer. Seal with adhesive cover and incubate at 4°C overnight.
4. Wash 3X with wash buffer, each time incubating for 1 min at room temperature.
5. Serially dilute the antigen standards (1:100) in blocking buffer and add 30 µl in each well.
6. Incubate at room temperature for 30 min - 1 hour.
7. Wash 3X with wash buffer, each time incubating for 1 min at room temperature.
8. Dilute the biotinylated detection antibody in blocking buffer (0.1 – 2 µg/ml) and add 30 µl in each well.
9. Incubate at room temperature for 45 min
10. Wash 3X with wash buffer, each time incubating for 1 min at room temperature.
11. Add 30 µl of 1X streptavidin to each well
12. Incubate at room temperature for 45 min
13. Wash 4 times with Buffer B incubating 30 seconds during each wash step.
14. Add 30 µl of 1X biotinylated DNA to each well and incubate at room temperature for 45 min.
15. Wash with Buffer B 7 times incubating 5 min during each wash step.
16. Wash with PBS twice incubating 1 min during each wash step.
17. Add 30 µl of 1X PCR mix to each well. Seal with a PCR film and transfer to PCR cycler with an optical compression pad.

Carry out the program as mentioned below:

Time	Temperature	cycles
5 min	95 °C	1X
30 s	50°C	28X
30 s	72 °C	
12 s	95 °C	

Note: Set up FAM (emission at 518 nm) as the fluorophor and tetramethylrhodamine as the quencher.

Results and Data Analysis

- Calculate threshold cycles (Ct). The instrument automatically calculates the threshold cycle (Ct), which represents the first PCR cycle at which the fluorescent reporter signal (dR) exceeds the signal of a given uniform 'threshold', manually set it in the phase where signal increases linearly (typically 100–3,000). Use a half-logarithmic plot of log dR against cycle number to choose the correct threshold value. Alternatively, use the threshold suggested by the instrument software.

☐ Export the Ct signals to an appropriate program for further data analysis (such as Microsoft Excel).

- Calculate dCt signals by subtraction of the maximal number of cycles (CMax) and the Ct value (e.g., for CMax = 40, dCt = 40–Ct).

☐ For each standard and unknown sample analyzed in duplicate, calculate mean values and s.d. of dCt.

- For quantification, perform either a linear regression of the appropriate detection range of the calibration curve CC1–CC7 (plot the dCt signals against the log spiked concentration for linear correlation) or use a nonlinear regression (e.g., 4-point fit) for the complete calibration curve. If a specific detection window is analyzed in several assays, the calibration curve should be adapted for linear correlation between signals and log concentration for all seven CC samples. The resulting equation is used for the quantification of unknown samples and the calculation of recovery rates of spiked samples.

Note:

The assay sensitivity could be increased by digesting the biotinylated DNA with BamH1 after step 15 in Assay procedure (A BamH1 restriction site is included in the biotinylated DNA)
Treat each well with 30 ul BamH1 in its reaction buffer and incubate at 37°C for 1-2 hours.
Transfer 2-5 ul of the above digested DNA in to a 96-well qPCR plate and perform the reaction (final volume 20-30 ul) as mentioned in the step 16.