4titude® Release Note

Issue: **09/2016**

Topic: **New Product Release**

Products: **Low Binding Plates for sensitive applications such as Next Generation Sequencing sample preparation**

Summary: Low binding polypropylene tubes allows for multiple incubation and transfer steps without sample loss

1. Overview

Polypropylene (PP) is the best plastic material for PCR tubes as PP is chemically inert, resistant to solvents, and well suited for injection moulding - allowing for production of thin-walled tubes for optimum PCR results.

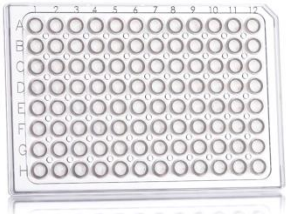
DNA has been shown to bind to PP tubes especially at high ionic strength, despite the very hydrophilic nature of this material (Gaillard & Strauss 1998*). Different PP polymers are used for the production of PCR consumables and as they differ in their characteristics including surface charges, they consequently bind DNA in varying amounts.

DNA binding to PP surfaces has typically only been an issue for reaction tubes and storage vessels but not for PCR/qPCR tubes. DNA sticking to tube walls is either released during denaturation steps and/or remains accessible for amplification. Nevertheless, due to a progressing volume miniaturisation and with new technologies such as Next Generation Sequencing (NGS), PCR/qPCR tubes are more and more also recommended for other applications.

NGS protocols often recommend PCR/qPCR plates for various incubation steps during sample prep and library construction. With the trend to a lower sample input, DNA loss through adhesion to PP surfaces must be avoided for these applications and researchers are consequently asking for low-binding tubes.

Unfortunately the term “low-binding” is not well defined. Many commercially available low-binding products are offered with little to no description of this feature - not to mention a lack of convincing data.

4titude now launches a range of PCR/qPCR plates featuring specially tested PP tubes with ultra-low DNA binding properties documented by convincing experimental data.



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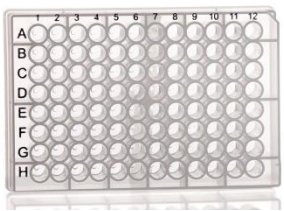

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**Avoiding adsorption of DNA to polypropylene tubes and denaturation of short DNA fragments. Gaillard, C & Strauss, F Technical Tips Online Vol 3 63-65 1998*

2. Experimental data

2.1 Binding of linear DNA to different PP polymers

2.1.1 Experimental set-up

A tenfold DNA dilution series was created containing a linear 1176 bp product (digested from plasmid ACTA1, TA cloned into the pGemT vector) resulting in DNA concentrations of 10 ng/µl, 1 ng/µl, 0.1 ng/µl, and 0.010 ng/µl.

50 µl of each DNA concentration were applied to a PCR plate and incubated for 30 minutes at 37°C. The DNA was afterwards transferred to the next row of the PCR plate for additional 30 minute incubation. This procedure was repeated seven times so that the DNA was incubated in 8 different tubes for a total of 240 minutes. All transfer steps were performed with commercially available low-binding tips (Corning).

2 µl of each DNA concentration were subsequently subjected to qPCR analysis and compared to 2 µl of the original dilution series (total DNA input = 2 ng to 0.002 ng per qPCR run) using ACTA1 primer and probe set designed by IdT (Integrated DNA Technology).

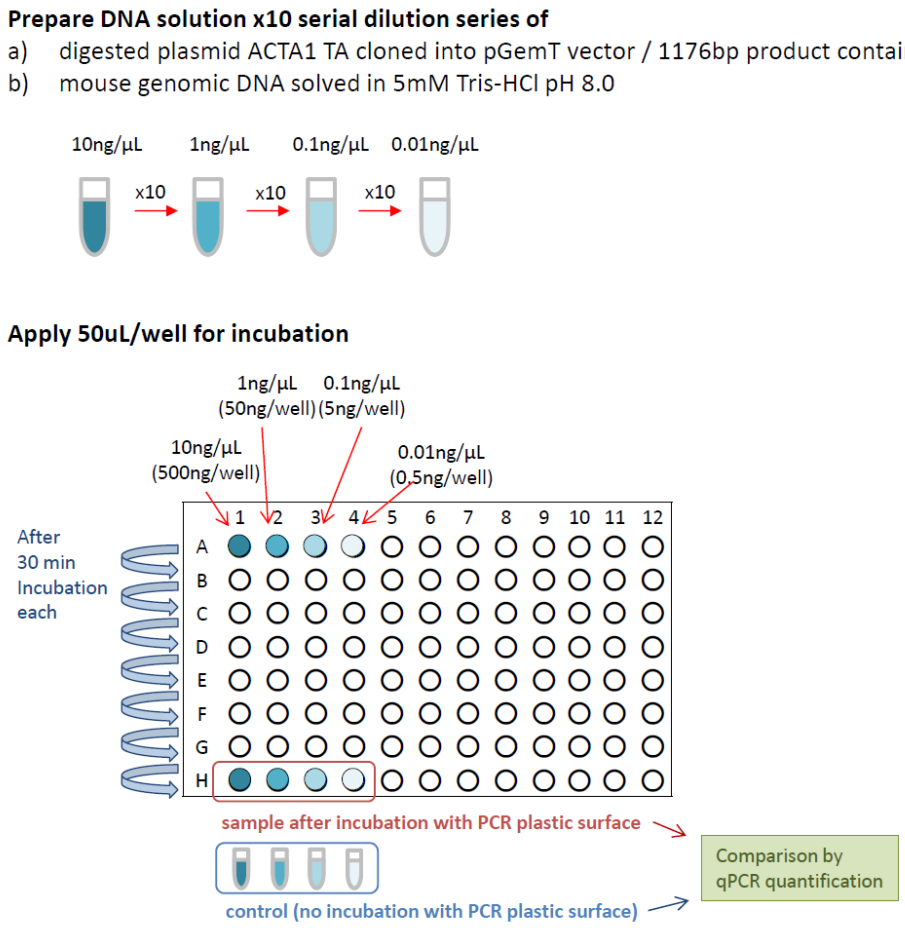


Figure 2.1 Binding of linear DNA to different PP polymers - Experimental set-up

2.1.2 Results

Three different PCR plates were compared:
a) a PCR plate with tubes made of the 4titude low-binding PP polymer
b) a PCR plate with tubes made of an alternative PP polymer and
c) a commercially available low-binding plate from competitor E.

Figure 2.2 shows Ct values obtained from the different DNA concentrations. The blue line shows the results for the control DNA not incubated in PCR tubes, the red line shows the results for DNA incubated in PCR tubes as described. The ΔCt is shown in green.

The red and the blue line should be identical (with ΔCt = 0) for plastic material which does not bind any detectable traces of DNA (minor differences may result from pipetting inaccuracies).

The 4titude low-binding material (a) shows almost an ideal pattern, the only small deviation from optimum results is a minor ΔCt of 0.44 cycles for the lowest DNA concentration of 0.002 ng/µl.

The alternative in-house PP polymer (b) tested in parallel revealed a significant DNA binding with a ΔCt of up to 2.57 cycles for the lowest DNA concentration. A surprisingly similar pattern with a ΔCt of up to 2.92 cycles was received from commercially available “low-binding” PCR plates from Competitor E (c).

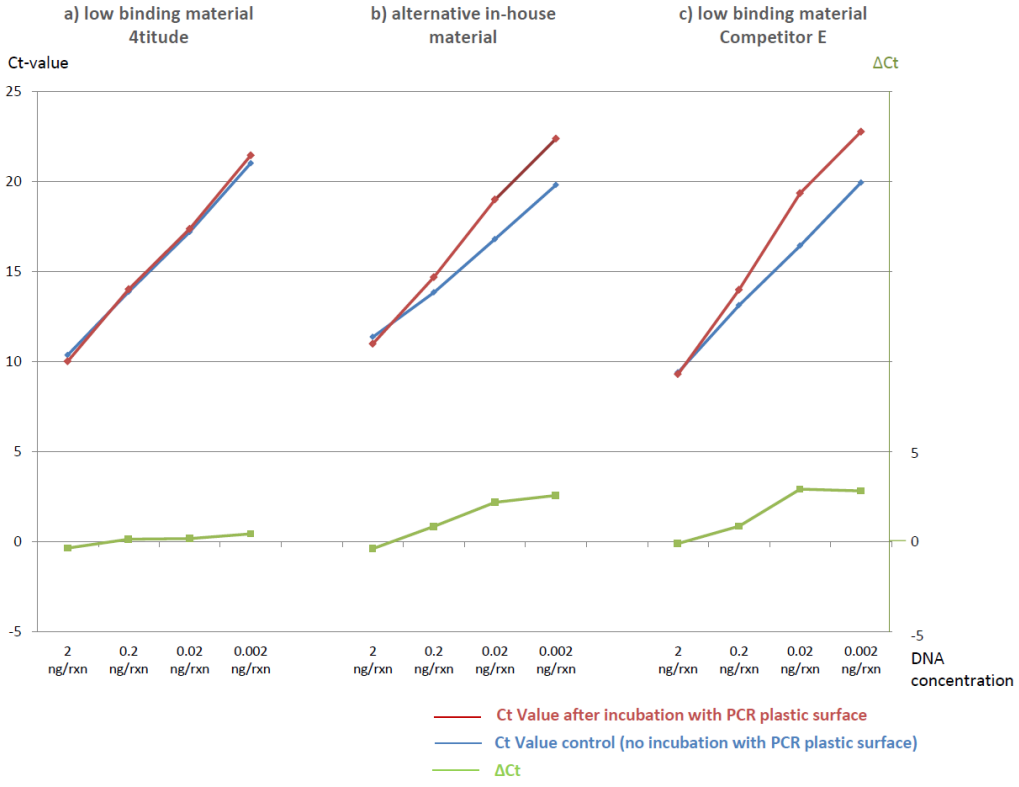


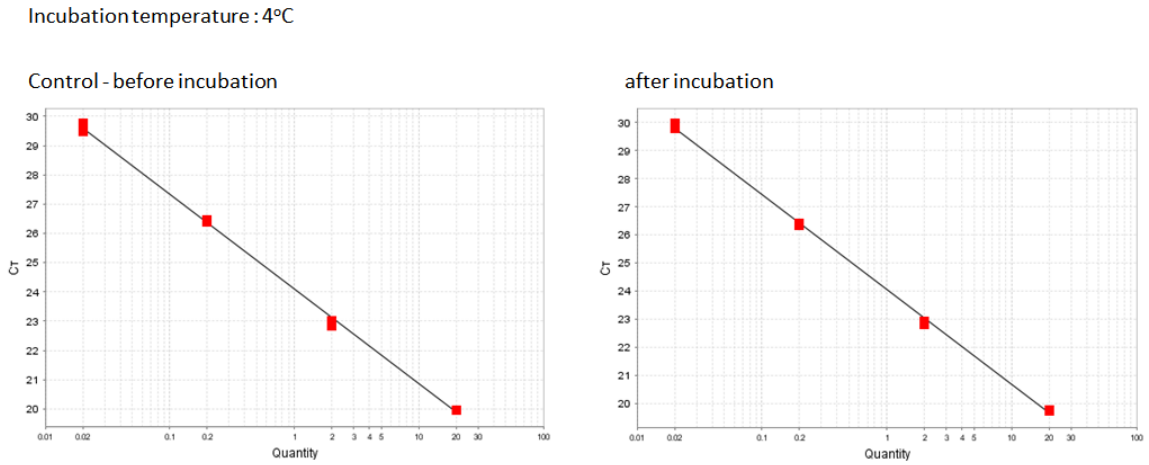
Figure 2.2 Binding of linear DNA to different PP polymers - qPCR comparison

The results clearly show the superior performance of the 4titude low-binding PP polymer indicating the advantages for sensitive applications such as Next Generation Sequencing sample preparation.

2.2 Binding of genomic DNA to low binding PP polymer at different temperatures

Further experiments were performed with the 4titude low-binding PP polymer. The experiment described above was **repeated with identical experimental conditions** using mouse genomic DNA (dissolved in 5 mM Tris-HCl pH 8.0) instead of the ACTA1 fragment testing three different incubation temperatures (4°C as a typical storage temperature for reaction mixtures, 37°C as a typical temperature for enzyme reactions, and 65°C as a typical temperature for enzyme denaturation). qPCR comparisons were performed with identical DNA concentrations using KAPA SYBR Fast qPCR Master Mix.

Figure 2.3 shows an exemplary standard curve taken from the 4°C incubation showing the Ct value for 4 different input concentrations for incubated samples versus controls. The results for other temperatures were comparable.



	before incubation	after incubation
Slope	-3.245	-3.277
R^2	0.999	0.999
Eff%	103.314	97.76

Figure 2.3 Binding of genomic DNA to low binding PP polymer at different temperatures - Exemplary standard curves

Table 2.1 shows the Ct values for all different experimental conditions - confirming almost no differences between samples incubated in 8 different tubes for a total of 240 minutes and the control not subjected to PCR plastic surfaces.

This data set verifies the superior performance of the 4titude low-binding PP polymer under different experimental conditions.

Incubation temperature : 4°C

	Ct value (without incubation)	Ct SD	Ct value (after incubation)	Ct SD	ΔCt
20 ng / rxn	19.97	0.01	19.77	0.01	-0.2
2 ng / rxn	22.93	0.08	22.87	0.05	-0.06
0.2 ng / rxn	26.44	0.03	26.38	0.05	-0.06
0.02 ng / rxn	29.61	0.14	29.86	0.10	0.25

Incubation temperature : 37°C

20 ng / rxn	19.85	0.02	19.64	0.05	-0.21
2 ng / rxn	22.93	0.06	22.77	0.05	-0.16
0.2 ng / rxn	26.28	0.03	26.37	0.05	0.10
0.02 ng / rxn	29.49	0.45	29.57	0.36	0.08

Incubation temperature : 65°C

20 ng / rxn	18.51	0.04	17.78	0.01	-0.73
2 ng / rxn	21.41	0.003	21.26	0.02	-0.16
0.2 ng / rxn	24.69	0.13	24.51	0.03	-0.18
0.02 ng / rxn	28.57	0.23	28.15	0.56	-0.42

Table 2.1 Binding of genomic DNA to low binding PP polymer at different temperatures - Ct values for all different experimental conditions

Experimental data: the data comparing DNA adhesion at different temperatures was kindly provided by our Japanese cooperation partner Nippon Genetics.

3. Sales strategy

3.1 Typical customers

Typical customers are either already using low binding consumables (for example tubes) and want to use the same quality for plates as well (which only Eppendorf and us currently offer) or customer are worried about low sample concentration in one of the key applications listed below.

3.2 Applications

- NGS DNA library construction
- Low volume PCR/qPCR
- Real-time PCR with ultra-low sample concentration

The three low binding products launched with this note are suitable for most qPCR instruments and typical NGS protocols. However, additional products with ultra-low binding properties may be added on request.

4. Sales arguments summary

- Low-binding feature results from selected low-bind polymers, no coating is used to achieve the binding characteristics

  → No contamination or modification of the samples

- Tested under a broad temperature range

  → Maximum DNA recovery after low temperature storage and high temperature incubation

- No DNA loss during incubation steps and sample transfer in NGS sample prep and library construction

  → Ideally suited for sensitive applications with ultra-low DNA input

When do I recommend the standard product and when the low binding version?

DNA sticking to tube walls is not a problem for **PCR/qPCR** as the DNA is either released during denaturation steps and/or remains accessible for amplification

→ The standard versions work fine!

For applications like **NGS sample prep** and **library construction**, various incubation steps and transfer steps from one tube to another are involved and DNA loss through adhesion to PP surfaces must be avoided

→ The low-binding versions are recommended!

Please note: "low binding" only relates to nucleic acids binding to the surface which has nothing to do with the behaviour of drops (low retention).

5. Main competitors

There are a number of companies offering low binding tubes including Axygen/Corning (MaxyMum Recovery tubes), Ambion (NonStick), Nunc (Bank-It vials) and Eppendorf, but the main competitor for plates is Eppendorf with the twin.tec DNA LoBind Plates:

Code	Description	Quantity	Exemplary end user price	4titude alternative
0030129504	Eppendorf twin.tec PCR Plates 96 LoBind, semi-skirted, clear	25		4ti-LB0770/C
0030129512	Eppendorf twin.tec PCR Plates 96 LoBind, skirted, clear	25		4ti-LB0960/RIG

(*reference: https://www.fishersci.de/shop)

6. Ordering and distributor price information

Code	Description	Quantity	Distributor Price
4ti-LB0770/C	FrameStar® 96 Well Semi-Skirted PCR Plate, ABI® Style, Low Binding	50	
4ti-LB0960/RIG	FrameStar® 96 Well Skirted PCR Plate, Extra Rigid, Low Binding	50	
4ti-LB0125	96 well Deep Well Storage Microplate, for use with magnetic separators, Low Binding	50	