FRAMEstar®
2-Component PCR Plates

FrameStar® two-component technology reduces evaporation from PCR plates, improving results and allowing for volume reductions to save on expensive reagents.

FrameStar® PCR plates maximise thermal stability at high temperatures. This prevents sample loss by minimising thermal expansion during PCR. The two-component design combines the advantages of thin wall polypropylene (PP) tubes, for optimum PCR results, with a rigid polycarbonate skirt and deck for highest thermal stability and rigidity. In contrast to standard polypropylene PCR plates, evaporation, particularly from corner positions and outer rows of wells, is minimal due to improved sealing properties. This allows for downscaling of reagent volumes and therefore saves costs.

- Two-component technology reduces thermal expansion and sample evaporation leading to improved consistency of PCR results.
- Well spacing and position post-PCR remain accurate so liquid handling devices can reliably add or remove the smallest quantities from the plate.
- Ideal for robotics, as plate distortion is eliminated post-PCR.
- FrameStar® is ideal for assay miniaturisation due to rigid frame eliminating warping, giving better sealing properties.
- Cost saving due to downscaling of reaction volumes.
- Lack of warpage reduces variation of fluorescent signals in optical assays, such as qPCR.

Benefits

- Improved PCR results
- Warp free
- SBS footprint maintained pre- and post-PCR
- Decreased sample evaporation, particularly from corner wells
- Improved seal integrity
- Recommended for low volume PCR
- Thin wall design for fast PCR
- Reliable use with stackers and liquid handling robots
- Guaranteed well integrity
- Free from bacterial and human genomic DNA, RNases and DNases
- Clean-room produced
- Barcoding and colour options

FrameStar® PCR plates are covered by one or more of the following U.S. patents or their foreign counterparts, owned by Eppendorf AG: US Patent Nos. 7,347,977 and 6,340,589

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"FrameStar® plates led to significantly better results and reduced evaporation compared to standard PCR plates."

Dr. Andreas Dahl, MPI f. Molekulare Genetik, Berlin, Germany

Thermal expansion of polypropylene (PP) plates leads to evaporation from outer wells.

PP is the optimum material for PCR tubes. It provides the most efficient heat transfer, as well as an inert surface with low binding capabilities for nucleic acids, proteins and other molecules. However, the material is not thermally stable in a plate format and expands and contracts during each PCR cycle (Figure 1). Such thermal expansion will weaken the plate seal and leads to sample evaporation, mainly from corner wells and outer rows.

Thermal cycler blocks do not prevent thermal expansion of PCR plates.

PCR blocks do not support PCR plates from the sides and the high temperatures from the thermal block and heated lid accelerate expansion of the polypropylene plates (Figure 2).

FrameStar® 2-component technology reduces thermal expansion and sample evaporation

The polycarbonate frame of FrameStar® plates is more heat resistant than standard polypropylene plates which reduces thermal expansion to a minimum. For this reason seal integrity remains intact even at elevated temperatures during PCR.

To illustrate the advantage of our two-component technology we have compared evaporation from standard PP plates and FrameStar® PCR plates: Each well of a non-skirted 96well plate (PP) and a Framestar® non-skirted design plate (code 4ti-0710) was filled with 10µl H2O. The plates were sealed with a qPCR adhesive seal (code 4ti-0560) and the weight of each plate was measured before and after performing PCR (30 cycles x 15'' 95°C; 15'' 55°C).

Table 1 shows that the average volume loss from standard PP plates using adhesive sealing was 2.3µl per well, which is equal to 23% of the total reaction volume. In contrast the volume loss from FrameStar® plates was only 0.49µl per well, so less than 5% of the total reaction volume.

Plate Type	Starting weight	Weight post PCR	Weight loss	Volume loss total/per well
Framestar® 4ti-0710	26.678g	26.631g	0.047g	47µl/0.49µl
Standard	17.807g	17.586g	0.221g	221µll/2.3µl

Table 1: Weight and volume loss from 96well PCR plates. Results shown are averages from 5 plates of each plate type. Standard PP plates showed more than 4 times higher volume loss than FrameStar® plates.

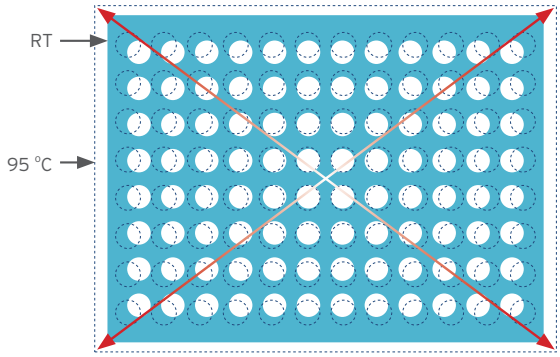

Figure 1: Standard plates with polypropylene frame expand by up to 2mm during thermal cycling which leads to movement of wells away from the plate centre. This movement is most significant in corner positions and outer rows of the plate. Sealing sheets do not expand at this rate so that the movement of wells will weaken the seal and lead to evaporation especially in corner positions and outer rows.

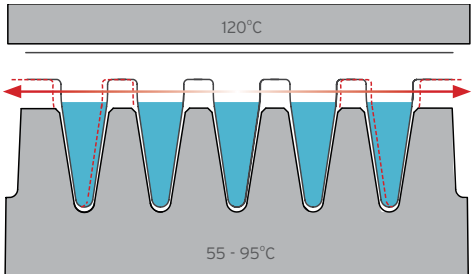

Figure 2: Side-on view of a PCR plate in a thermal cycler. The sealed plate is sandwiched between the cycler block and the heated lid but it is only partly fixed in position at the bottom of tubes, allowing the plate to expand horizontally.

Evaporation from standard PP plates is highest in the outer wells

Since thermal expansion and movement of wells in standard PP plates is enhanced around the edges of the plates (see Figure 1) evaporation is highest from the two outer rows of wells. Figure 3 illustrates the levels of sample evaporation from different areas of PP plates. Only the inner 32 wells of a standard 96well plate show low levels of evaporation, sample loss is high from the two outer rows which contain more than 65% of the wells.

FrameStar® 2-component plates improve consistency of PCR results

We have compared the degree of evaporation from different areas of standard PP and FrameStar® PCR plates. First, the 64 outer wells (two outer rows) of both plate types were filled with 10µl H20, plates were then sealed with a qPCR adhesive seal (code 4ti-0560) and their total weight determined before and after PCR. The experiment was repeated with a set of plates of which the inner 32 wells (green area in Figure 3) were filled. Table 2 shows that evaporation from outer wells of standard PP plates was significant and 65% higher than from inner wells. In contrast, FrameStar® plates showed low levels of sample loss across the plate.

The results below show that reaction volumes remain consistent across the 96wells (or 384wells, data not shown) in FrameStar® plates. In contrast, the reaction volumes in standard plates differ increasingly between wells during PCR. Buffer concentrations in outer rows will increase dramatically and result in reduced enzyme activity. In extreme cases samples will fully evaporate.

FrameStar® PCR plates minimise sample loss across the plate

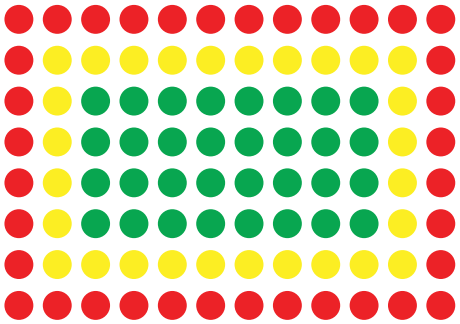

Figure 3: Evaporation from the outer rows (red) of a standard PP PCR plate is highest, medium level evaporation occurs in the second row (yellow) and sample loss from the inner 32 wells is lowest.

Weight and Volume loss

Plate Type	Starting weight (g)	Weight post PCR (g)	Weight loss (g)	Volume loss Total	Volume loss Per well
Framestar® 4ti-0710 outer 64 wells	26.230	26.193	0.037	37µl	0.57µl
Standard PP, outer 64 wells	17.299	17.118	0.181	181µl	2.8µl
Framestar® 4ti-0710 inner 32 wells	25.841	25.824	0.017	17µl	0.53µl
Standard PP, inner 32 wells	17.132	17.078	0.054	54µl	1.69µl

Table 2: Weight and volume loss from different sections of 96well PCR plates. Results shown are averages from 5 plates of each plate type. Volume loss from the outer wells of standard PP plates was 5-times higher than from FrameStar® plates.

FrameStar® 2-component technology allows for reduction of assay volumes and cost

Due to the much improved seal integrity reaction volumes can often be reduced when using FrameStar® plates. Such downscaling of experiments can be successfully implemented without any loss of assay sensitivity or consistency (Figure 4) and reagent savings can be considerable.

Improved Sealing

FrameStar® technology provides a much-improved level of seal integrity: During PCR, with heat seals or adhesive seals, the excessive thermal expansion/contraction of standard polypropylene plates, can have a damaging effect on seal quality. This is especially evident around the edge of the plate where these forces are at their maximum resulting in significant sample evaporation from the outer wells. In contrast, the higher thermal stability of FrameStar® plates results in a reliable seal in all areas of the plate during PCR and in cold storage down to -80ºC.

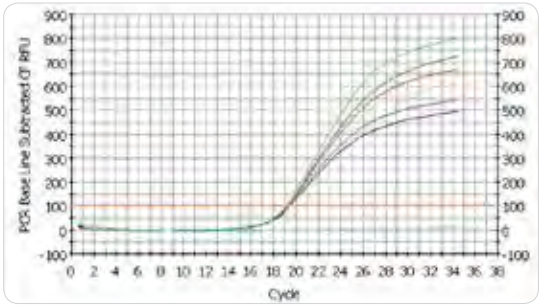

Figure 4: Comparable Ct Values and mean deviations were obtained with 15µl and 25µl SyBr Green assays in heat sealed FrameStar® plates.

Plate Distortion Post-PCR

	X-axis	Y-axis	Warpage
FrameStar® 96, skirted	0.02mm	0.02mm	0.03mm
Standard 96well PP Plate	1.18mm	1.69mm	1.8mm
FrameStar® 384	0.02mm	0.02mm	0.03mm
Standard 384well PP Plate	1.22mm	1.82mm	2.2mm

Distortion tests have been performed with different PCR profiles using a variety of sealing materials. The results shown were obtained using the following protocol with 5µl reaction volumes, plates sealed with adhesive seal sheets (4titude® code 4ti-0500), in a Thermo Px2 Cycler: 1min 94ºC, 30x(30sec 94ºC, 30sec 55ºC, 30sec 72ºC), 5min 72ºC, holding step at 4ºC. Averages from measurements of 10 plate samples are shown. X- and Y- axis measurements were taken in the middle sections of the plate skirt. Warpage was measured as a maximum deviation from a flat plane.

Sealing Options

4titude® offers a wide range of sealing options for FrameStar® plates:

- A variety of optical, pierceable and peelable heat sealing options, for manual and automated use (sheets and rolls respectively).
- Adhesive films and foils available including materials for qPCR and other fluorescence based applications.
- Domed or flat optical cap strips for all 96well plates.

Detailed information on all sealing options can be found on pages 60 to 83.

4titude® offers a range of adhesive and heat sealing films and foils. The plate above has been sealed with Q-Stick™ Adhesive for qPCR, code 4ti-0565

4titude® offers domed cap strips and optically clear flat cap strips. The plate above has been sealed with flat cap strips, code 4ti-0751

Barcoding

All skirted and semi-skirted FrameStar® plates are available barcoded for identification and traceability. Please see page 54 for more details.

Fluorescence Based Analysis

For experiments involving fluorescence detection, e.g. qPCR, we have developed plates which are especially low in autofluorescence. For this type of experiment we recommend to use our plates with white wells to maximize signal intensity due to the reflective properties of the pigment.

Reduced Risk of Cross Contamination

Raised rims around each tube aid sealing and prevention of cross contamination between samples.

Two FrameStar® application notes are available:

- Miniaturization of a qPCR assay with reduced reagent volumes to achieve cost savings in gene expression analyses in rumen epithelium.
- Consumable effects on low volume TaqMan® assays for quantitative PCR on Roche LightCycler® 480.

These can be downloaded from the Technical section of our website: **www.4ti.co.uk**

Colour Options

FrameStar® plates are available with purple, blue, red, green, clear and black frames, all with clear tubes. In addition we offer plates with white tubes optimised for fluorescence assays. Plates with black tubes are also available.

If the plate you require is not available, please enquire.

Printed Alphanumeric Grid Reference

For easier sample identification, all our PCR plates are supplied with a printed alphanumeric grid reference.

Plate Compatibility

FrameStar® plates are available in various formats compatible with most PCR cyclers, real-time detection systems and sequencers.

See pages 46 to 48.

FrameStar® plates have been validated on most automation platforms. Please contact 4titude® for further details.