PIPETTE TIPS

Why RPT?

Useful information from STARLAB

TipOne RPT

Repel Polymer technology
Pipette tips

STARLAB

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Why **RPT**?

You know how it is: a Lysis buffer needs to be prepared and, annoyingly, the Triton X-100 cannot be pipetted properly. You are also experiencing problems with the pipetting of Tween or glycerine? You can, of course, pipette very, very **slowly**, but many users have resorted to **cutting the pipette tips** to create a larger opening. However, not only is this complicated it can also lead to contamination.

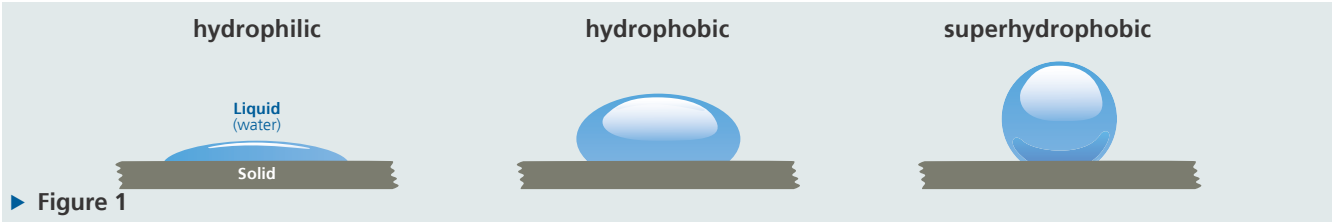
There are now **tips with wide openings** available in the market, but they won't help with very sticky substances with a high viscosity and low surface tension. **Reverse pipetting** is one solution to increase pipetting accuracy, however, this method requires a larger quantity of reagent which may not be available.

It is not only the preparation of solutions but also the handling of many samples that requires pipetting with maximum precision. These are expensive enzymes or samples which are only available in very small quantities as well as samples which have a tendency to generate foam. The quantity of liquid which remains in the pipette tip should be reduced as much as possible in these cases. However, frequently the liquids do not drain completely from the pipette tip; individual droplets or even a thin film remain in the tip. Using pipette tips with special surface properties offers a practical solution to this problem. The objective is to create a surface that is as hydrophobic and smooth as possible to ensure that no liquid is retained in the tip. In this context, this is also referred to as **"Low Retention" tips**.

What to do?

What does **hydrophobicity** actually mean?

Hydrophobic means "water repellent" and describes substances which do not mix with water and usually let it "run off" on surfaces. **Hydrophilic** substances, on the other hand, are "water-loving". The differences can be described using the shape of a droplet as a result of surface tension:

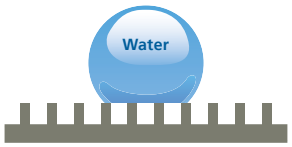


▶ **Figure 1**

On superhydrophobic surfaces (e.g. the "lotus effect", Fig. 2) water assumes a nearly spherical shape. This means that the contact area between the liquid and the surface is only very small. The droplet cannot stay there and runs off.

▶ **Figure 2** Lotus effect

If you look closely, superhydrophobic surfaces have a pronounced surface structure with protrusions in nanometre and micrometre sizes. This leads to the formation of a stable air layer underneath the droplet (Fig. 3).



▶ **Figure 3** Schematic of a water droplet on a superhydrophobic surface

The bottom of the droplet is held on the protrusions by the surface tension of the water. The surface tension (for solids: surface energy) may vary greatly with different materials (Fig. 4)

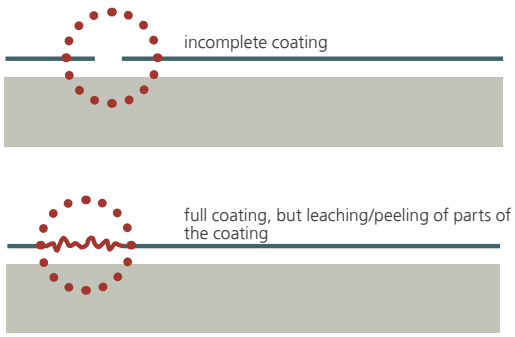
Material	Surface tension [mN/m]
Water	72
Untreated polypropylene (PP)	30
Paraffin wax	26
Silicone	22
PTFE	19
TipOne® RPT pipette tips	6 - 10

▶ **Figure 4** Surface tension of different substances

The lower the surface energy, the more hydrophobic the material will be. Pipette tips are made from polypropylene (PP). When designing "Low Retention" pipette tips, the objective is to reduce the surface tension energy of the PP to reduce the amount of liquid remaining in the tip by the increased hydrophobicity. There are three major manufacturing technologies to make plastic surfaces as hydrophobic as possible:

"Wet chemical" methods

The tip is immersed in a medium which leaves a film on the surface that causes the effect. A standard approach is the use of siliconized consumables. During siliconization, the tip is coated with a thin layer of silicone (Fig. 5). Siliconized materials are also used in other industries, e.g. for consumer products (tarpaulins) or in cosmetics (silicone is added to hair care products).
Many scientists used to siliconize tubes themselves in their laboratories, but today these tubes are readily available in the market.



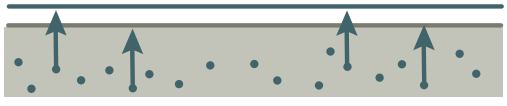
▶ **Figure 5** Solid body with silicone layer

Advantages:
Can be performed in-house.
Economical for the user and/or manufacturer.

Disadvantages:
If the coating process is not completed, there may be areas which do not have any or an incomplete silicone layer (e.g. the inside of small volume tips). Liquid droplets may form in these areas and reduce the effect. The coating may not be stable and, depending on the liquid that is pipetted, it may even be washed out in places, causing the silicone to "bleed" into the sample. A negative impact on the test result cannot be excluded completely if siliconized pipette tips are used. Silicone may, for instance, inhibit the Taq Polymerase in PCR reactions [2]. For fluoridation, fluoropolymers are used. This requires the purchase of the corresponding reagents (e.g. "FluoroPel") for the pipette tips which then form a type of "Teflon" layer. The coating may not be stable and, depending on the liquid that is pipetted, it may even be washed out in places causing the fluoride to "bleed" into the sample. A negative impact on the test result cannot be excluded completely if fluoridated pipette tips are used. Fluorides may inhibit enzyme reactions [3]. The "Low Retention effect" disappears after the contact with acetone or it weakens.

Adding additives

To avoid the disadvantages of the coating method a different technology was developed. For this method, additives are added to the PP before injection moulding. These are generally types of wax. During the manufacturing process they migrate to the surface and form a wax layer which provides a hydrophobic surface (Fig. 6).



▶ **Figure 6** Solid with wax layer

Advantages:
It is not a coating method and therefore provides a complete surface. Simple, quick and economical for the manufacturer.

Disadvantages:
The wax layer may not be stable and, depending on the liquid that is pipetted, it may even be washed out in places causing the wax to "bleed" into the sample. A negative impact on the test results can therefore also not be excluded completely with this technology. Wax may have a negative impact, e.g. on PCR reactions [1]. Autoclaving frequently leads to the disappearance of the "Low Retention" effect. The material then looks milky or cloudy and the wax layer can often be scratched off with a fingernail.

Repel Polymer Technology (RPT)

Most companies use siliconization, fluoridation and the formation of a wax layer for the manufacture of "Low Retention" tips.
All have one major drawback: as an additional layer is formed on the surface of the PP which is not firmly connected to the PP at an atomic level there is, for instance, a risk of bleeding and, consequently, contamination of the sample.

In contrast, the "Repel Polymer Technology" (RPT) by STARLAB optimises the plastic surface using molecular hydrophobising (Fig. 7). The entire tip has the same excellent properties and has a 3x lower surface energy than Teflon (PTFE). This complex and highly innovative method creates an invisible superhydrophobic surface which cannot bleed due to its covalent bond.

This prevents contamination or negative cross reactions in experiments. It also produces less foam.

How does **RPT** work on a molecular level?

- Irregular structure without orientation
- Low percentage of superhydrophobic end groups on the surface = surface tension varies
- Physical adhesion of the coating
- Deteriorating performance if organic solvents (e.g. acetone) are used, caused by the solubility of the coating
- Thick coating varies
- Performance varies from one tip to another

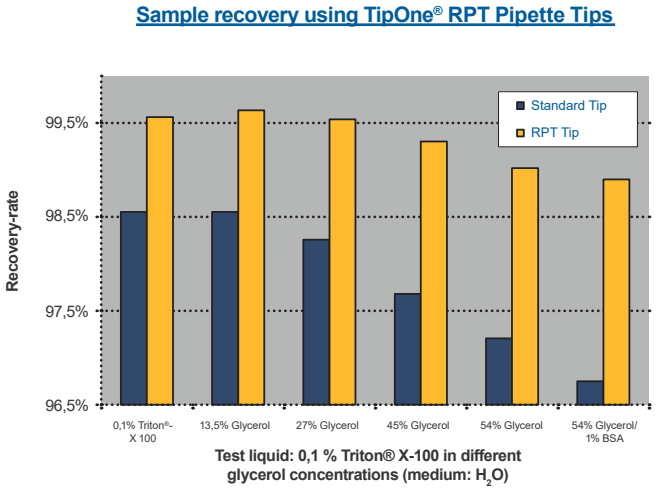
- Chains are oriented away from the surface in a regular manner
- High percentage of superhydrophobic end groups on the surface = very low and evenly distributed surface tension
- Covalent bond of the chain segments to the PP substrate
- Consistent performance including the use of organic solvents (e.g. acetone) due to the covalent bond with the substrate
- Evenly distributed low thickness of the superhydrophobic surface
- Consistent performance from one tip to another

Figure 7 Differences between conventional "Low Retention" and TipOne® RPT tips.

TipOne® RPT vs. standard tip

To find out how TipOne® RPT tips perform when compared to standard tips, different solutions with liquids with "critical" flow properties were prepared and pipetted using 200 µl RPT tips and/or standard tips. It was determined how much liquid runs back out of the tip (Fig. 8)

Figure 8 Sample recovery of TipOne® RPT Tips with standard pipette tips ▶



When TipOne® RPT tips are used, sample losses are lower. The more viscous/concentrated the pipetted liquid, the greater the difference is between standard tips and RPT tips.

Impact on the **"Low Retention"** effect through acetone treatment

TipOne® RPT tips*:

▶ **Figure 9** Impact on the "Low Retention" effect through acetone treatment for TipOne® RPT tips.

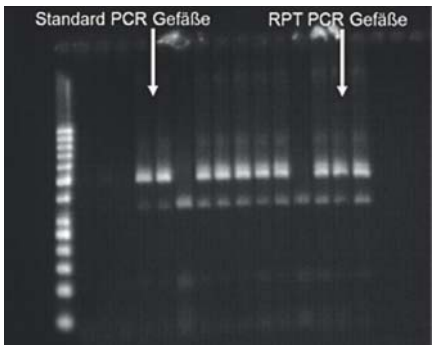
Competitor N*:

▶ **Figure 10** Impact on the "Low Retention" effect through acetone treatment on a competitor's tips.

While there is virtually no impact on the "Low Retention" effect with RPT tips following the acetone treatment, competitor N's tips show virtually no "Low Retention" effect after the acetone treatment. The experiment shows how unstable the "Low Retention" surface of this competitor's tips are.

Impact of the **"Low Retention"** technology on PCR reactions

As described above, there are indications that substances which form the hydrophobic surface when conventional "Low Retention" tips are used may have a negative impact on PCR reactions. Consumables manufactured using Repel Polymer technology do not have any impact on PCR, shown here using tubes:



▶ **Figure 11** Agarose gel electrophoresis

A PCR system was selected which is very sensitive to PCR inhibitors: human Beta-actin (5H/6H) & HotMaster Taq DNA polymerase. The PCR was performed in standard 0.2 ml PCR tubes and 0.2 ml RPT-PCR tubes. For this test, a special small series of PCR tubes with an optimized surface was produced using RPT technology. The amplified DNA quantity in tubes with RPT treatment is comparable to the DNA yield in standard PCR tubes.

Conclusion:

Scientific experiments need to deliver measurable results. They must be reproducible, repeatable and objective. In routine diagnostics (e.g. medicine or food), this applies even more. Laboratories go to great lengths to prevent the causes of errors such as incorrect storage of samples or insufficient mixing from the outset or to at least minimise errors. In addition to the usual sources of error there are two more which many people do not know or which are often simply ignored: sample loss or contamination by the pipette tips. Especially in applications where particularly valuable and, in some cases, irrecoverable samples are pipetted (e.g. in drug discovery and in the proteomics sector), the loss of samples results in high costs.

By choosing TipOne® pipette tips with highly innovative RPT technology you can avoid the loss of samples and eliminate contamination. TipOne® RPT tips offer a higher precision and fewer losses during the pipetting of samples which contain detergents (e.g. Triton X-100, Tween or SDS):

- "Mastermixes" for standard techniques in molecular biology such as (q)PCR, restriction digest and ligations
- Preparation of DNA standards for gel electrophoresis
- Proteomics

They also produce less foam. Consumables which have been optimised with Repel Polymer technology do not have a negative impact on PCR reactions.

You don't know how the "Low retention" tips you are currently using work and how reliable they are? Ask your supplier and also request test results!

By the way: TipOne® RPT tips are also always **"DNA Low Binding"**, making it very difficult for DNA molecules to stick to the tip and therefore keeping the DNA sample loss to a minimum. This means that RPT tips are ideal for pipetting DNA.

***Material & method test with acetone**

Devices: Electronic 12-channel pipette

Chemicals: Test liquid: Green food dye McCormick (contains H_2O, propylene glycol, FD&C yellow, FD&C blue 1, propyl paraben) acetone ≥99.8% (p.a.)

Procedure:

12 tips are weighed and then attached to the pipette. The tips are filled with the test liquid up to the nominal volume (200 µl) and then slowly emptied (speed 3). After three seconds the tips are emptied using a blow-out step to remove the residual liquid that has formed around the tip opening. The tips are photographed immediately after pipetting.

To test the stability against organic solvents, the tips are filled with 200 µl of acetone three times and then dried in a vacuum (0.2 mbar) at 30 °C for two minutes. This is followed by the test using green food colouring as described above.

Sources:

[1] Hans-Jürgen Butt, Günter K. Auernhammer, Doris Vollmer, Oberflächen mit Phobie, Physik Journal (2015), 25

[2] Rimantas Kodziusa, Kang Xiaoc, Jinbo Wud, Xin Yid, Xiuqing Gongd, Ian G. Fouldsb, Weijia Wena, Inhibitory effect of common microfluidic materials on PCR outcome, Sensors and Actuators B 161 (2011) 349-357

[3] Karlheinz Graf, Fluoride unter umwelt(zahn)medizinischen Aspekten (II), Umwelt Medizin Gesellschaft 22 (2009) 75-76

www.starlab.click/rpt