Application Note

Detergent in Polystyrene ELISA

Peter Esser, M.Sc., Senior Scientist, Thermo Fisher Scientific

Detergent is used in ELISA for washing off loosely or unspecifically bound reactants. It may also be used for blocking possible excess solid surface (e.g. polystyrene) after coating with one reactant to avoid unspecific immobilization of subsequent reactants.

Often a detergent is used according to tradition and routine procedures, or it is arbitrarily adopted from one application to another.

However, detergent may be a double-edged sword and should be selected with care depending on the particular assay reactants and immobilizing surface material.

Introduction

Detergents are molecules consisting of a distinct hydrophobic and hydrophilic part (Table 1).

Their washing effect is based on the ability to disperse hydrophobic molecules in aqueous medium, i.e. to dissolve unstable hydrophobic bonds between surface and coating reactant, and unspecific hydrophobic bonds mutually between reactants on the surface.

Their blocking effect is based on the ability to compete with other molecules for both hydrophobic and hydrophilic binding sites.

However, an immobilized detergent may in itself affect further specific and unspecific immobilization characteristics of the solid phase, e.g. by applying hydrophilic groups to a hydrophobic surface, or by interfering with the active sites of reactant molecules.

The reversibility of possible detergent mediated solid phase alterations depends on the detergent binding strength, implying detergent size, charge and structure in relation to the other assay ingredients. Therefore, the use of detergent should be optimized for each separate application.

Method and Results

To elucidate some of the detergent conditions mentioned above, five detergents of various sizes and charges (schematized in Table 1) were tested in a catching antibody assay according to the procedure listed in Table 2. Thermo Scientific Nunc Immuno Modules F8 with physically adsorbing surfaces, i.e. partly hydrophilic MaxiSorp (Cat. No. 468667) and hydrophobic PolySorp (Cat. No. 469078) were used. The results are presented in Fig. 1.

Thermo SCIENTIFIC

Table 1

Schematic illustration of the five detergents used in the experiments. Tween 20 = ikosaoxyethylene sorbitan monolaurate (Merck 822184); Triton X-100 = octylphenoxy octaethoxy ethanol (Merck 8603); SDS = sodium dodecyl sulfate (Serva 20760); DTAB = dodecyltrimethylammonium bromide (Sigma D-8638); CHAPS = 3-[(cholamidopropyl) dimethylammonio]-1-propanesulfonate (Sigma C-3023). According to other sources, the hydrophilic polyoxyethylene part of Tweens is divided into three separate arms linked to the sorbitan part, and the hydrophobic octyl part og Triton X-100 is branched.

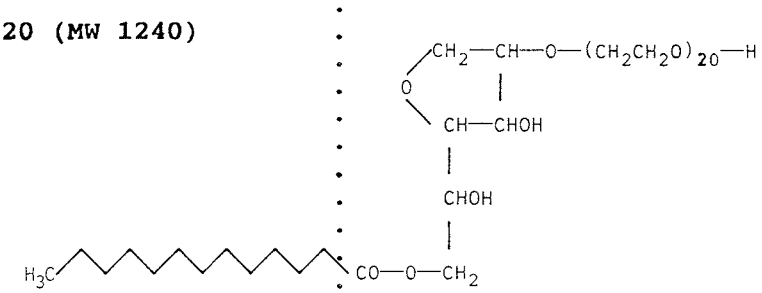
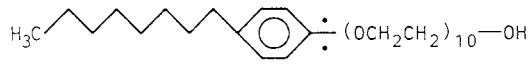
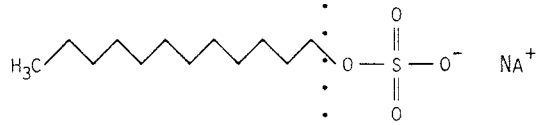
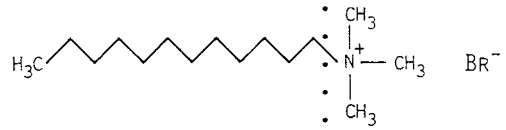
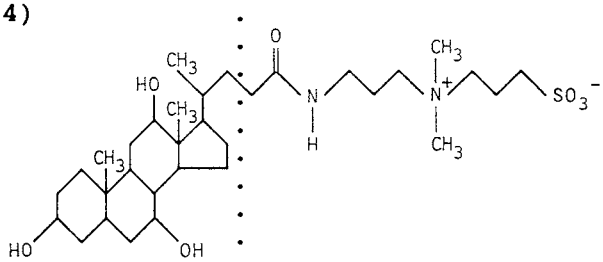
	Hydrophobic part	Hydrophilic part
Non ionic	**Tween 20 (MW 1240)**	
	Triton X-100 (MW 646)	
Ionic + or -	**SDS (MW 265+23)**	
	DTAB (MW 228+80)	
Zwitterionic	**CHAPS (MW 614)**	

Table 2

Procedure with MicroWell plates with MaxiSorp or PolySorp surfaces using each of the five detergents in the five code alternatives (bottom row), all in one experiment. The procedure was followed by HRP reaction using H_2O_2/OPD substrate. SaR = swine anti-rabbit antibody (Dako Z 196) = catching antibody; R:HRP = peroxid ase conjugated rabbit antibody (Dako P 128) = target conjugate; S:HRP = peroxidase conjugated swine antibody (Dako P 217) = indifferent conjugate.

Step	Reagent	Time	% Detergent added				
1st layer	SaR, 5 µg/mL in PBS or None	overnight	(0)	(0)	(0)	(0)	(0)
1st wash	PBS + 0.2 M extra NaCl	3x	0	.05	.05	.05	.05
2nd layer	R:HRP, 1.3 µg/mL in PBS or S:HRP, 1.3 µg/mL in PBS	2 hr	0	0	.05	0	.05
2nd wash	PBS + 0.2 M extra NaCl	3x	0	0	0	.05	.05
Detergent code used in Figs. 1-3			−−−	+−−	++−	+−+	+++

Discussion

From the results with the present test system several detergent effects relevant to ELISA can be observed (Fig. 1):

1. In general, the detergents exert a blocking effect against unspecific adsorption only if they are present together with the conjugates (++− and +++); only in these cases there are no significant unspecific signals.

2. Tween 20 makes an exception to statement 1. Its presence in the 1st wash seems to be sufficient for blocking unspecific adsorption in subsequent layers on both surfaces. This may be a consequence of its relatively large size, which presumably implies that it remains firmly bound to the surface, unlike the other detergents. However, its larger size is due merely to a larger hydrophilic part, wherefore it is difficult to explain its stable blocking effect on the hydrophobic Nunc™ PolySorp™ surface.

3. The positively charged DTAB exhibits large unspecific signals in all cases. Probably it binds to the conjugates, thereby facilitating their unspecific adsorption.

4. Tween 20 and DTAB seem to enhance the signals when used in the 2nd wash (+−+ and +++). This may be due to the presence of detergent remnants in the substrate solutions in those cases.
   In a control experiment with or without detergent added to the substrate solution after direct coating of the surfaces with HRP conjugate it was indeed observed, especially with MaxiSorp™, that Tween 20 and DTAB enhanced the substrate reaction; SDS and CHAPS somewhat reduced the reaction, whereas Triton X-100 was indifferent. There is no immediate explanation to these interferences with the substrate reaction.

5. Tween 20, Triton X-100, and in particular SDS give small specific PolySorp signals (compared with CHAPS), whereas only SDS gives relatively small signals with MaxiSorp. This may be explained by differences in washing effects between the detergents, SDS being the most harsh, combined with the fact that PolySorp binds less native antibody in a stable way than does MaxiSorp [1]. However, a consequence of this would be: the lower the specific signal, the higher the signal if the surface has only been cleared for loosely bound antibody by 1st wash detergent (+−−).
   This does not seem to be the case in general, so an additional inhibitory interference with the antibody specificities, especially by SDS, may be postulated in accordance with findings by others [2].

6. Without detergent (−−−) there seems to be no difference between the signals with specific and unspecific conjugate, nor between MaxiSorp and PolySorp.
   In a control experiment using ^{125}I labelled 1st layer antibody it was found that equal amounts of antibody remained on MaxiSorp and PolySorp when no detergent was subsequently used. This can explain the equality of specific signals on MaxiSorp and PolySorp by absence of detergent, but not the equally large unspecific signals. The latter may be explained by occurrence of a second-positioned, unspecific adsorption of conjugate in competition with specific binding.

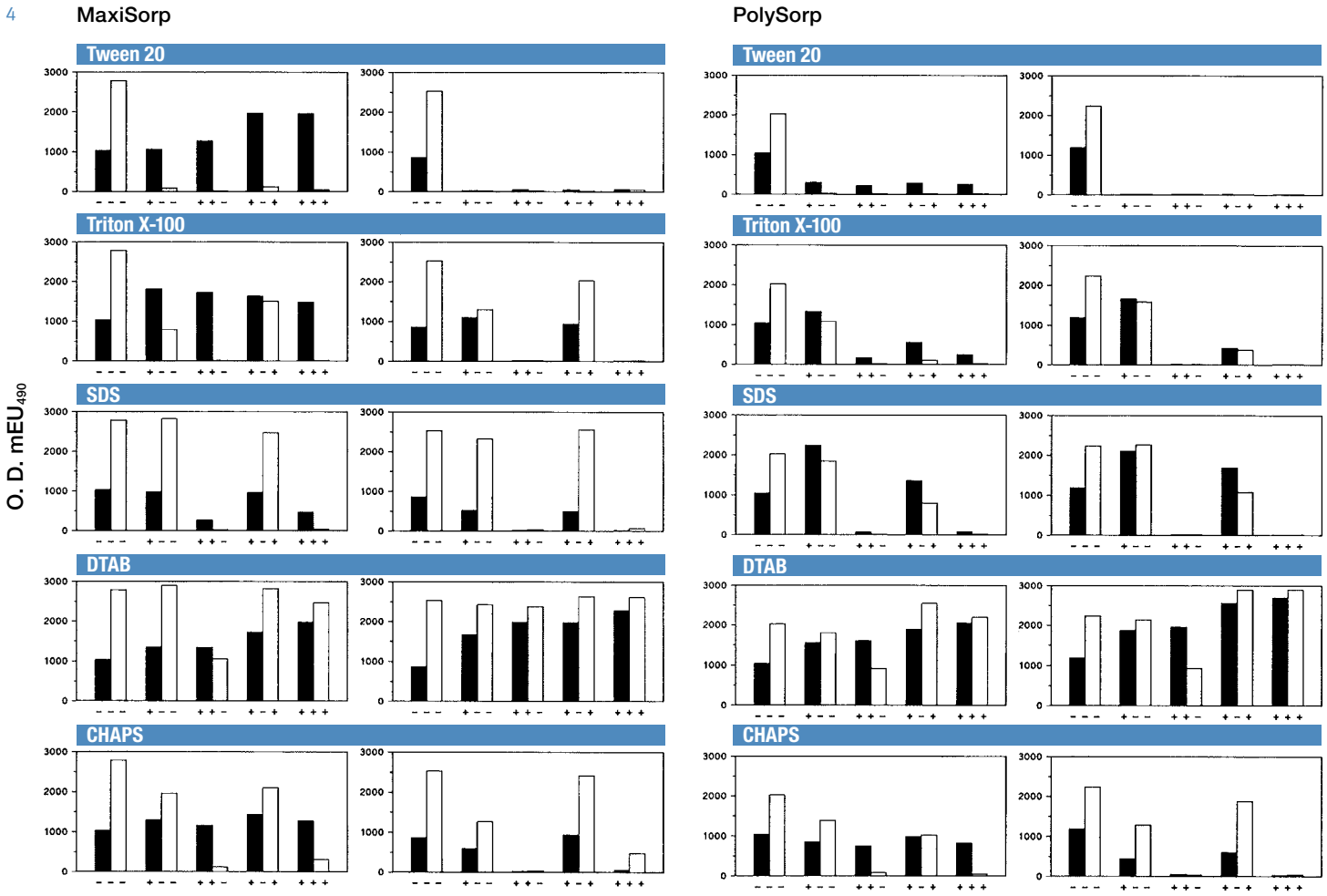


Fig. 1

Mean results with MaxiSorp (left diagram block) and PolySorp (right diagram block) from three independent experiments, each one with mutually comparable signals obtained according to the procedure in Table 1. Left diagrams (in MaxiSorp and PolySorp block respectively) show results for target conjugate; right diagrams show results for indifferent conjugate; ■ = with 1st layer; □ = without 1st layer.

A schematic explanation of the general detergent conditions is attempted in Fig. 2, which has given rise to the stoichiometric modelling in Fig. 3.

MaxiSorp after coating

Some loose binding sites have remained unoccupied, whereby an equal amount of specific sites have remained available.

After – – – : By absence of detergent no loosely bound antibody has been washed off, thus some specific sites have remained masked. Available specific and unspecific binding sites have competed for target conjugate binding.

After + + + (or + + –): By presence of detergent in every step all unspecifically bound antibody/conjugate has been washed/kept off, having left all specific sites available for target conjugate binding.

After + – – : By presence of detergent in the 1st wash only, the loosely bound antibody has been washed off, having unmasked all specific sites, which have been competing for target conjugate with some unspecific sites capable of conjugate binding.

After + – +: Same as after + – –, because all unspecifically bound conjugate has been firmly bound, possibly through the enzyme (|).

PolySorp after coating

Same as MaxiSorp, except that more antibody has been loosely bound at the expense of firmly bound antibody.

After – – – : Same as MaxiSorp, except that unspecifically bound conjugate has only been loosely bound, possibly through the enzyme (+).

After + + + (or + + –): Same as MaxiSorp, except that more loosely bound antibody/conjugate has been washed/kept off, having left less antibody on the surface for target conjugate binding.

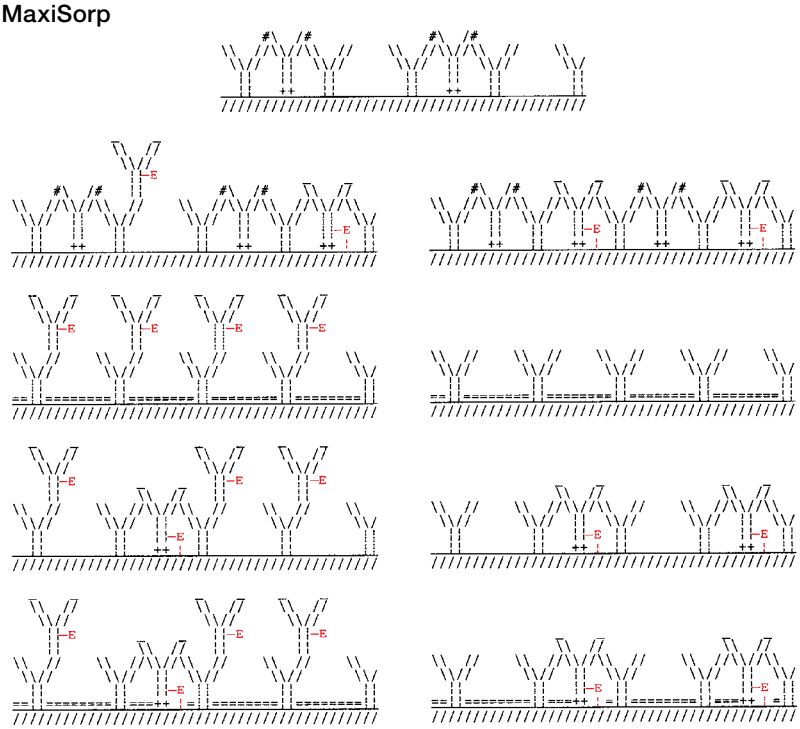
After + – – : Same as MaxiSorp, except that more space has been available for unspecific binding of conjugate, some of which has been firmly bound, possibly through the enzyme (T).

After + – +: Same as after + – –, except that loosely bound conjugate has been washed off by presence of detergent in the 2nd wash.

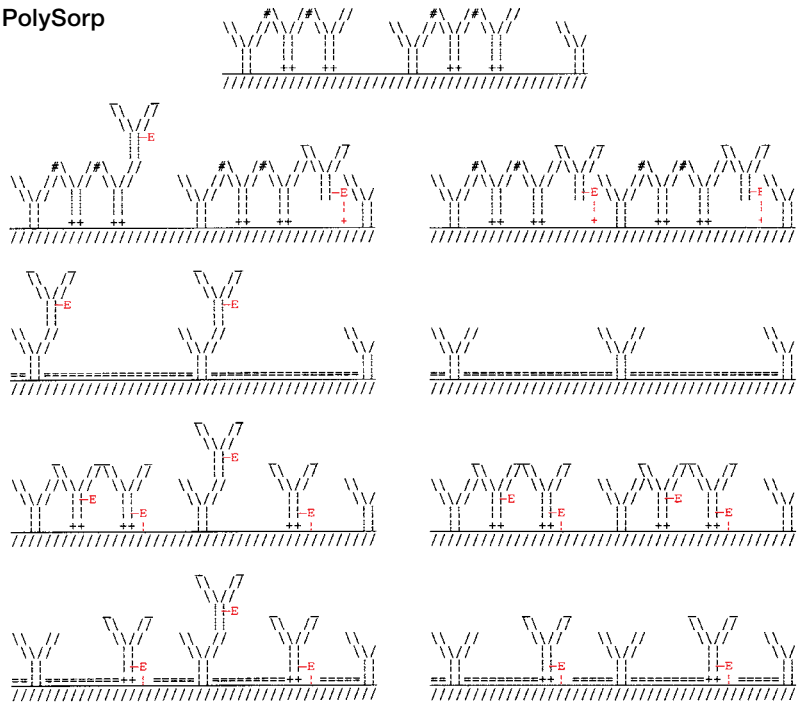
Fig. 2

Schematic explanation of the results in Fig. 1 for MaxiSorp (above) and PolySorp (below) with special reference to the Triton X-100 results. Left and right diagrams illustrate the situations with target and indifferent conjugates, respectively; Y-shapes represent antibodies; Y-E represents enzyme conjugated antibody whose non-involved specific sites are indicated by the small »closing« lines above the arms. The overall idea is that unless detergent is subsequently used (= =) some coating antibody will be loosely bound (++) in a secondary position between the firmly bound antibody, resulting in mutual sterical hindrance (#) of antibody specificities. Consequently, for spatial reasons, the implied secondary, unspecific binding sites are assumed to compete with the specific sites for target conjugate binding by absence of detergent. For simplicity, only the right arm antibody specificities are considered.

MaxiSorp



PolySorp



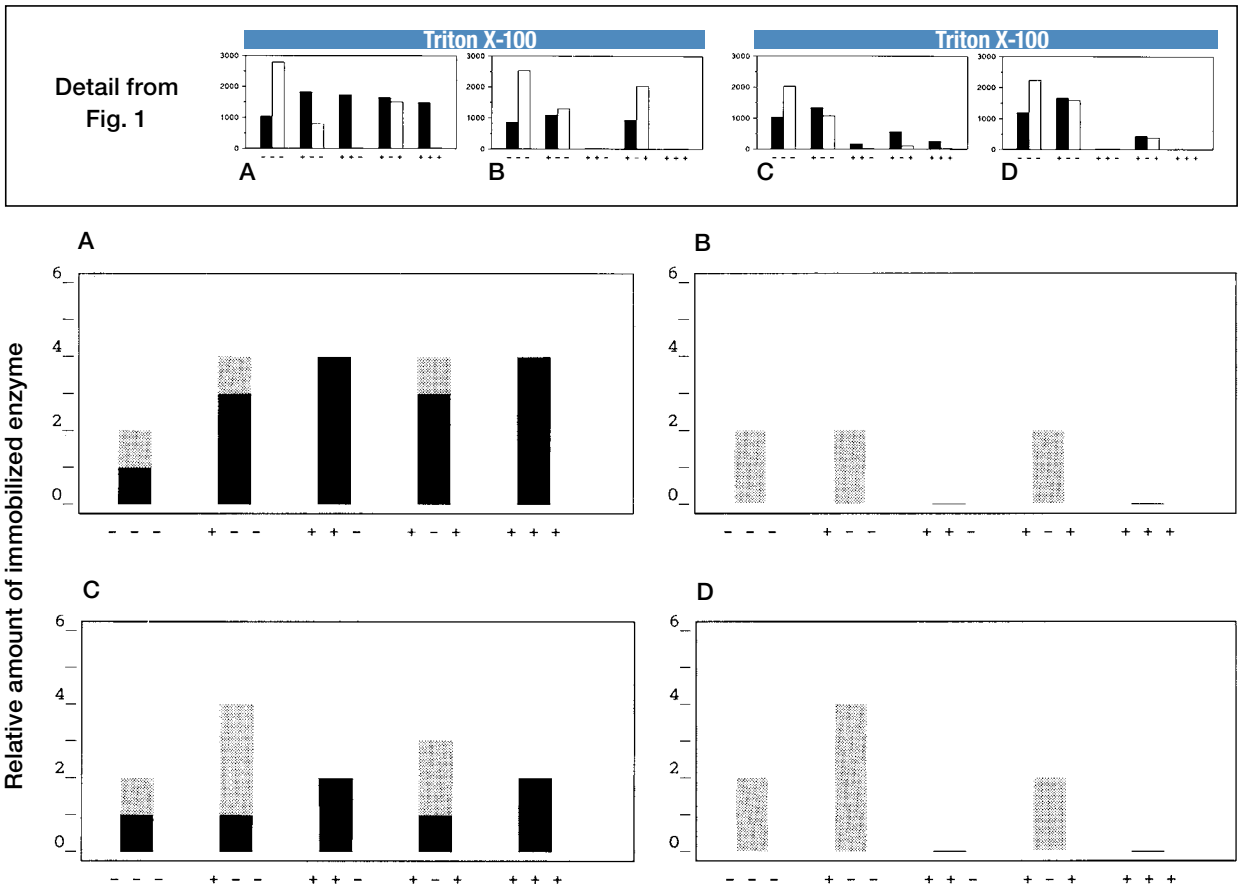


Fig. 3

Stoichiometric model, based on counts of immobilized enzyme in Fig. 2, of the results for coated surfaces, i.e. with 1st layer, resembling most closely the results with Triton X-100 (filled columns in above detail) A & B: MaxiSorp; C & D: PolySorp; A & C: with target conjugate; B & D: with indifferent conjugate; ■ = specific part of signals; ■ = unspecific part of signals.

Conclusion

From this investigation some general guidelines concerning the use of detergent in ELISA can be extracted:

1. Detergent is necessary for washing off loosely adsorbed reactant to abolish sterical hindrances caused by reactant crowding on the surface.

2. If no other blocking agent is used, detergent must be present during incubation with post-coating reactants to avoid unspecific adsorption. Tween 20 is an exception, as it performs a stable blocking once applied, like typical blocking agents such as BSA or casein.

3. Detergents with net charges like SDS and DTAB must be avoided because of their disadvantageous interferences with the assay reactants.

4. Among the investigated detergents, Triton X-100 or Tween 20 seem to be optimal for application with the MaxiSorp surface, whereas the apparently more gentle CHAPS may be the best choice with PolySorp.

This investigation does not give a complete picture of the detergent conditions with ELISA. Important aspects, such as detergent effect dependence on concentration and pH, or detergent performance in concert with typical blocking agents, must wait to be addressed at a later time.

References

1. Esser P. (1988).
   Principles in adsorption to polystyrene.
   Thermo Scientific Nunc Bulletin No. 6, 1-5.

2. Crumpton M.J. & Parkhouse R.M.E. (1972).
   Comparison of the effects of various detergents on antigen-antibody interaction.
   FEBS Letters 22, 210-212.

Application Note

thermoscientific.com/oemdiagnostics

ANZ: Australia: 1300 735 292, New Zealand: 0800 933 966; **Asia:** China Toll-free: 800-810-5118 or 400-650-5118;
India: +91 22 6716 2200, India Toll-free: 1 800 22 8374; Japan: +81-3-5826-1616; Other Asian countries: 65 68729717
Europe: Austria: +43 1 801 40 0; Belgium: +32 2 482 30 30; Denmark: +45 4631 2000; France: +33 2 2803 2180;
Germany: +49 6184 90 6000, Germany Toll-free: 0800 1-536 376; Italy: +39 02 95059 554;
Netherlands: +31 76 571 4440; Nordic/Baltic countries: +358 9 329 10200; Russia/CIS: +7 (812) 703 42 15;
Spain/Portugal: +34 93 223 09 18; Switzerland: +41 44 454 12 22; UK/Ireland: +44 870 609 9203
North America: USA/Canada +1 585 586 8800; USA Toll-free: 800 625 4327
South America: USA sales support: +1 585 899 7198 **Countries not listed:** +49 6184 90 6000 or +33 2 2803 2000

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

TILSPNUNCBU08 0314