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Application Note

Comparison of Greener Detergents in Mammalian Cell Lysis & Downstream Applications

Introduction

Detergents are commonly used reagents in biochemical applications. One of the most frequently employed is Triton™ X-100, a non-ionic surfactant often used to solubilize proteins. It is considered a comparatively mild detergent, non-denaturing, and is utilized for lysing cells to extract protein and cellular organelles. According to the EU REACH Regulation, the use of alkylphenol ethoxylates including Triton™ might require an authorization. For more information, please download our Statement on the Inclusion of Alkylphenol Ethoxylates in Annex XIV **SigmaAldrich.com/REACH-Detergents**

In this application note, the capabilities of alternative detergents for effective mammalian cell lysis and downstream applications were assessed and compared to detergents commonly used: IGEPAL® CA-630, Triton™ X-100 and Triton™ X-114. Given the widespread use of Triton™ X-100 in lab applications, it is important to identify alternative detergents which allow effective mammalian cell lysis, and will not affect downstream assays.

Materials & Methods

All materials were obtained from us unless otherwise specified.

Cell Preparation and Lysis

Cells were lysed using Sigma-Aldrich's Mammalian Cell Lysis Kit **(MCL1)**. The cell lysis buffer was prepared as per manufacturer's instructions, except alternative detergents were used to substitute for IGEPAL® CA-630.

Detergent mixes (final concentrations):

- 50 mM Tris-HCl, pH 7.5, 1 mM EDTA **(T8815)**
- 150 mM NaCl **(S4684)**
- 0.1% Lauryl sulfate, sodium salt **(L1787)**
- 0.5% Deoxycholic acid, sodium salt **(D4437)**
- 1% Protease inhibitor cocktail [4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), pepstatin A, bestatin, leupeptin, aprotinin and trans-Epoxysuccinyl-L-leucylamido(4-guanidino)butane (E-64)] **(P8340)**
- 1% Experimental [TERGITOL™ 15-S-9 **(15S9)**, TERGITOL™ 15-S-7 **(15S7)**, TERGITOL™ 15-S-40 **(STS0003)**, ECOSURF™ EH-9 **(STS0012)**] or control detergent [IGEPAL® CA-630 **(I2653)**, Triton™ X-100 **(T9284)**, Triton™ X-114 **(93422)**]

CHO-S cells expressing an IgG1 protein were lysed according to the MCL1 kit protocol for cells in suspension. Supernatants were removed and tested in downstream DNA and protein applications. Two biological replicates of each sample type were prepared.

DNA Quantification

CHO DNA in the prepared supernatants was quantified using the PicoGreen™ Assay and by qPCR. The Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen, P7589) was used according to manufacturer's instructions. For qPCR, the 50,000-fold dilutions of the supernatants were prepared by serial dilution, and the DNA was quantified using the resDNASEQ™ Quantitative CHO DNA Kit (ThermoFisher, 4402085) according to manufacturer's instructions.

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Protein Applications

Bicinchoninic Acid (BCA) Protein Assay

Quantification of total protein in the supernatants was carried out using BCA Protein Assay Kit (Pierce, 23225), according to manufacturer's instructions.

Protein A HPLC

Protein A HPLC (ThermoFisher, 1502412) was used to determine the IgG1 concentration in each sample.

SDS-PAGE and Western Blot

SDS-PAGE was carried out under both reducing and non-reducing conditions. 10 µL of each sample, including an IgG1 reference sample, was loaded into wells of BioRad 4-15% gels (#456-1084). A molecular weight marker was also loaded (Bio-Rad Precision Plus Protein™ Unstained, 161-0363).

Four gels were run in total, two reducing and two non-reducing. For each condition one gel was stained overnight with Oriole™ Fluorescent Gel Stain (BioRad, 161-0496), before washing with Milli-Q® water, and imaging. The other was used for Western blotting. Following transfer to PVDF membrane using Trans-Blot® Turbo™ Mini PVDF Transfer Packs (Bio-Rad, 1704156), primary Anti-Human IgG1 (Sigma-Aldrich, SAB4200768) was used to detect the protein of interest.

Results

DNA Applications: To assess the potential for interference of each detergent in qPCR, a control experiment was performed in which each lysis buffers was spiked with 300 pg/mL of CHO DNA. There was no difference in the DNA concentration in the mixes containing detergent compared to the DNA spike control (Milli-Q® water with CHO reference DNA) (Fig. 1), with recoveries ranging from 92% to 100%. This indicates that there is no significant interference in the qPCR assay at the concentrations tested.

DNA concentration in the supernatants was determined via two methods, PicoGreen™ and qPCR (Fig. 2). The results were then normalized against the IGEPAL® CA-630 control. In the PicoGreen™ assay, DNA concentrations ranged from 83% to 103% of the IGEPAL® CA-630 control. Quantification of DNA in the supernatants via qPCR displayed larger differences in experimental conditions, with ECOSURF™ EH-9 86% higher than the IGEPAL® CA-630 control (Fig. 2). Other conditions ranged from 11% to 29% greater than the IGEPAL® CA-630 control condition. Of note, TERGITOL™ 15-S-40 conditions were not included in this analysis due to difficulties with sample handling following cell lysis.

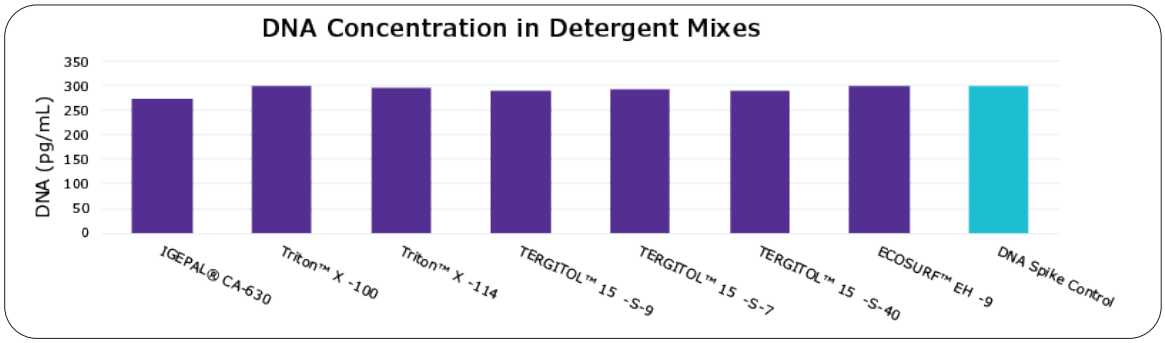

Figure 1. Quantification of DNA in detergent mixes (no cells).
Detergent mixes and a Milli-Q™ water control were spiked with 300 pg/mL CHO reference DNA, and qPCR was used to determine the DNA concentration in the mixes.

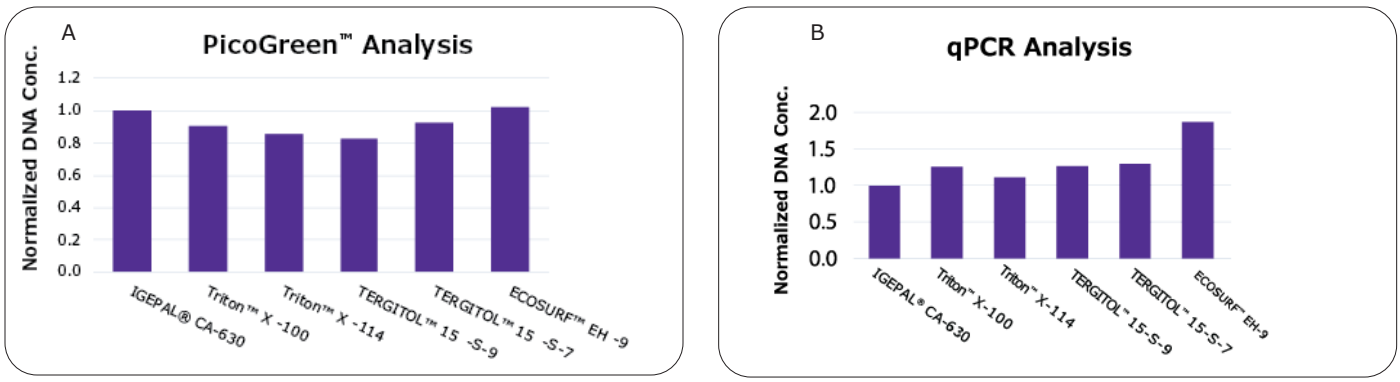

Figure 2. Quantification of DNA in lysed cell supernatant preparations. DNA concentrations in each sample were normalized against the IGEPAL® CA-630 control. A. PicoGreen™. B. qPCR.
*Data not shown: TERGITOL™ 15-S-40 lysis buffer.

Protein Applications

Protein concentration in the supernatants was determined using two different assays, the BCA assay and Protein A HPLC. The results were normalized against IGEPAL® CA-630 control for comparison. Quantification via the BCA assay showed consistent total protein recovery with values ranging from 88% to 108% of the IGEPAL® CA-630 control. The concentration of IgG1 in the supernatants was determined via Protein A HPLC. All the samples gave comparable results, except for the sample lysed with buffer containing TERGITOL™ 15-S-7, which had significantly decreased IgG1 concentration in one replicate **(Fig. 3)**.

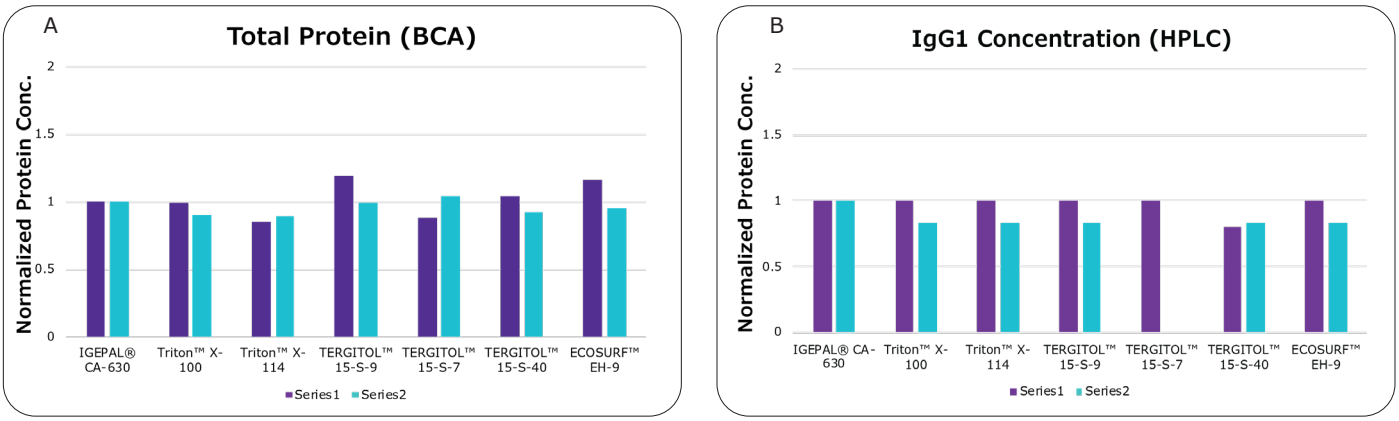

Figure 3. Quantitation of protein in lysed cell supernatant preparations.

A.The total protein concentration in the supernatants from the cells lysed with the detergent mixes was determined using the BCA assay. These results were then normalized against the IGEPAL® CA-630 control. Both replicates shown.

B.The IgG1 protein concentration in the supernatants from the cells lysed with the detergent mixes was determined via HPLC. These results were then normalized against the IGEPAL® CA-630 control. Both replicates shown, with the exception of TERGITOL™ 15-S-7, for which the second replicate resulted in total sample loss.

Visual examination of the stained SDS-PAGE gels indicated that the protein band pattern from the supernatants was similar across all the lysis buffers except TERGITOL™ 15-S-40, which showed slight differences in the band pattern. All the lanes contained the same bands as the control IgG1. These bands were seen at the 150 KDa mark in the non-reducing gel, corresponding to intact IgG1, and at 25 KDa and 50 KDa in the reducing gel, corresponding to the light and heavy chains respectively.

Visual examination of the Western Blots showed that bands the same size as the IgG1 control were detected across all supernatants, in both the non-reducing and reducing gels. Although differences in the band pattern of the supernatant from the TERGITOL™ 15-S-40 sample were observed in the SDS-PAGE gel, this was not reflected when detecting the IgG1 reference in the Western Blots.

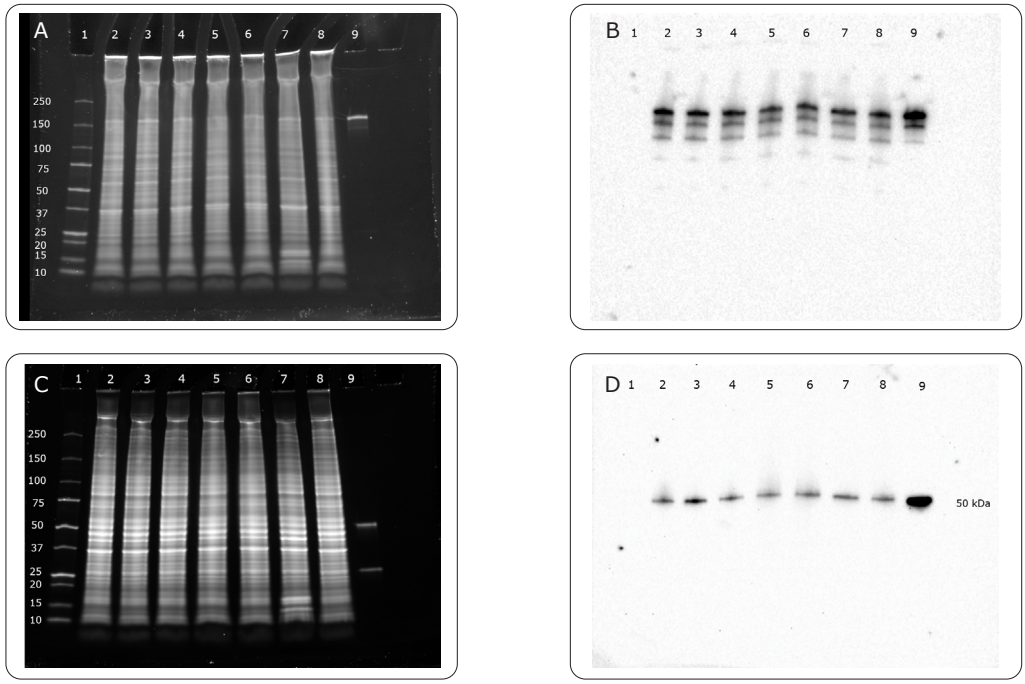

Figure 4. Total cell lysates on SDS-PAGE gels. 10 µL of each sample was loaded into wells of 4-15 % SDS-PAGE gels, along with reference IgG1. [1) Marker, 2) IGEPAL® CA-630, 3) Triton™ X-100, 4) Triton™ X-114, 5) TERGITOL™ 15-S-9, 6) TERGITOL™ 15-S-7, 7) TERGITOL™ 15-S-40, 8) ECOSURF™ EH-9, 9) IgG1 standard]. A. Non-reducing SDS-PAGE gel stained with Oriole™ stain, B. Western Blot carried out on non-reducing SDS-PAGE gel, C. Reducing SDS-PAGE gel stained with Oriole™ stain, D. Western Blot carried out on reducing SDS-PAGE gel.

Discussion

The results of this study show that there are several detergents that may substitute for Triton™ X-100, Triton™ X-114 or IGEPAL® CA-630 in cell lysis buffers. While TERGITOL™ 15-S-40 appears compatible with protein and DNA quantification assays, it is not recommended for cell lysis applications due to the difficulty in sample handling, not only at 1% concentration but also at 0.5% and 2% (data not shown). The other detergents tested in this study – TERGITOL™ 15-S-7, TERGITOL™ 15-S-9 and ECOSURF™ EH-9 – worked well for cell lysis and were compatible with the downstream protein and nucleic acid applications in these examples.

The suitability of alternative detergents as a replacement for octylphenol ethoxylate containing detergents, like e.g. Triton X™-100, is only demonstrated in the context of this study. To date, other applications may still require the use of detergents containing octylphenol ethoxylate.

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