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A Geno Technology, Inc. (USA) brand name

CMC-535™ Detergent Assay

A Fluorescent Detergent Assay for Assaying Detergents Below Their CMC Values

(Cat. # DG535)

INTRODUCTION

The CMC-535™ Detergent Assay is a simple, fluorescent assay designed for the detection of various detergents in aqueous solutions and is an ideal compliment for the DetergentOUT™ detergent removal columns. The basic principle of the assay is the interaction of detergents with the CMC-535™ Fluorescent Dye, resulting in an enhancement of the fluorescent signal that is proportional to the detergent concentration.

The assay can be used to quantitate detergent levels with the use of a standard curves or can be used to compare detergent removal rates to a diluted starting material sample.

The assay is designed to detect detergents at concentrations below their CMC values for most detergents. The assay is compatible with most aqueous buffers, with the exception of buffers that contain phosphates, including molecules that release phosphates (i.e. ADP and ATP). As the assay is sensitive to molecules with strong hydrophobic segments, we recommend <1mg/ml protein and <0.1mg/ml nucleic acids.

The assay is suitable for 200 microwell assays.

ITEMS SUPPLIED

Description	For 200 Assays
CMC-535™ Fluorescent Dye [100X]	200µl
CMC-535™ Reagent 1	20ml
CMC-535™ Reagent 2 [100X]	100µl

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store at 4°C. The kit components are stable for 1 year when stored and used as recommended.

PREPARATION BEFORE USE

- Dilute the CMC-535™ Fluorescent Dye [100X] in CMC-535™ Reagent 1 100 fold. Prepare this the day of use and only prepare as much as required. You need 100µl for every assay. For 10 assays, add 10µl CMC-535™ Fluorescent Dye [100X] into 990µl CMC-535™ Reagent 1.

- Dilute the CMC-535™ Reagent 2 [100X] in pure water 100 fold. Prepare this the day of use and only prepare as much as required. You need 50µl for every assay. For 10 assays, add 5µl CMC-535™ Reagent 2 [100X] into 495µl pure water.

ADDITIONAL MATERIALS REQUIRED

- 0.1% Detergent Stock Solution for standards
- Black 96-well plates (Costar 3915 or Greiner 655076)

PROTOCOL

1. If using, prepare a detergent standard curve. Serial dilute the stock solution 1:1 six times to give standards of 0.1, 0.05, 0.025, 0.0125, 0.0063, 0.0031 and 0.0016%. We recommend performing the standards in duplicate. 50µl is required for each standard.

2. Add 50µl each standard, sample and DI water as a blank to a well.
   NOTE: *For samples with an expected detergent concentration higher than their CMC (see appendix), dilute with deionized water and use 50µl of diluted sample in the assay.*

3. Add 100µl 1X CMC-535™ Fluorescent Dye and mix on a plate shaker for 2 minutes.

4. Add 50µl 1X CMC-535™ Reagent 2 and mix on a plate shaker for 2 minutes.

5. Read the fluorescence at 535nm, using an excitation at 485nm. Plot the correlation between the fluorescence values and the detergent concentrations and compare the data from the unknown samples.

TROUBLESHOOTING

The principle of the CMC-535™ Detergent Assay makes it sensitive to molecules with strong hydrophobic segments. If the assay is producing unexpected data, we recommend attempting to remove other molecules with hydrophobic segments.

Protein Removal

We recommend protein concentrations <1mg/ml as a general guideline. Dilute samples to reduce protein concentration.

To remove proteins, we recommend using UPPA (Cat. # 786-122) to precipitate the protein away from the aqueous detergents. Use the CMC-535™ Detergent Assay on the supernatant. The protein can be resuspend if needed for further downstream applications

Nucleic Acids

We recommend nucleic acid concentrations <0.1mg/ml as a general guideline. Dilute samples to reduce nucleic acid concentration.

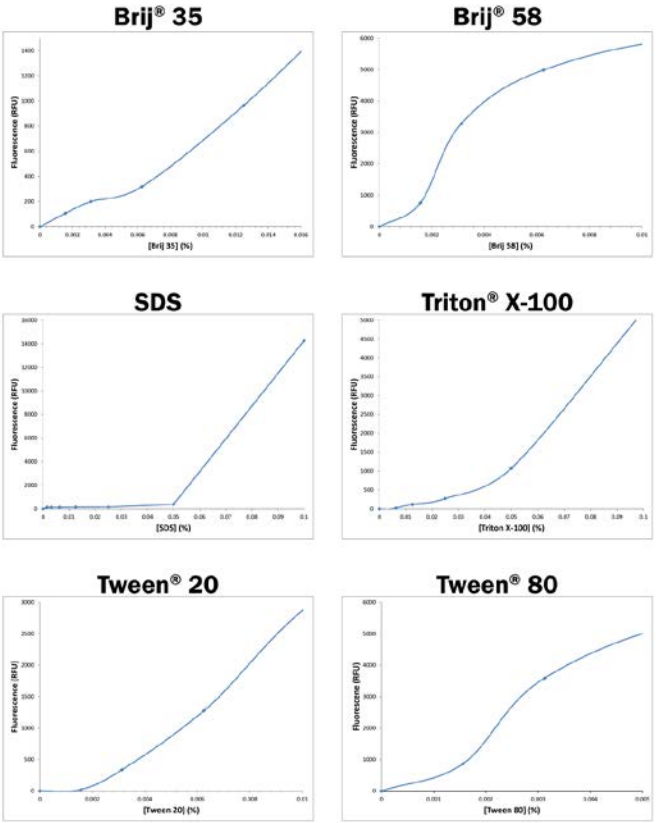
Treat the sample with nucleases to remove the nucleic acid interference.

APPENDIX 1: CMC (CRITICAL MICELLE CONCENTRATION) VALUES

Detergent	CMC
Tween® 20	~0.06x10^{-3}M
Tween® 80	~0.012x10^{-3}M
Triton® X-100	~0.2x10^{-3}M
Brij® 35	90µM
Brij® 58	7-77µM
SDS	7-10mM
CTAB	1mM

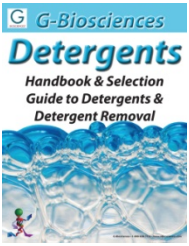
APPENDIX 2: EXAMPLE DETERGENT STANDARD CURVES

This are shown as examples, do not use these to calculate the concentration of your detergents as temperature, buffers and other factors will affect your standard curves.



RELATED PRODUCTS

Download our Detergents Handbook.



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