Technical Bulletin

Detergent Assay Kit

Catalog Number MAK459

Product Description

Detergents are surfactants that are amphiphilic, which means that they are partly hydrophobic and partly hydrophilic. Detergents fall into three categories: anionic, cationic, and non-ionic/zwitterionic. They are commonly used in household cleaning products, in gasoline as additives, and in biological reagents.

Simple, direct and automation-suitable procedures for measuring detergent concentration in biological samples are very useful. The Detergent Assay Kit is designed to measure detergent directly in samples without pretreatment. Above the critical micellar concentration, a detergent forms micelles in solution; these micelles trap the colorimetric dye in the assay reaction. The intensity of the color is directly proportional to the detergent concentration in the sample.

The linear detection range of the assay is:

- TWEEN® 80: 0.012 to 4 mM
- TWEEN 20: 0.06 to 5 mM
- Triton™ X-100: 0.23 to 12 mM
- Brij® L23/35: 0.09 to 5 mM
- DTAC (dodecyltrimethylammonium chloride): 0.08 to 2 mM
- SDS (sodium dodecyl sulfate): 10 to 25 mM.

The Detergent Assay kit is suitable for detecting traces of detergent in purified protein samples and the determination of detergent in water and soil samples.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Reagent A 20 mL
  For use with Cationic or Non-ionic Detergents
  Catalog Number MAK459A
- 100 mM Triton X-100 Standard 100 µL
  Catalog Number MAK459B
- Reagent B 20 mL
  For use with Anionic Detergents
  Catalog Number MAK459C
- 250 mM SDS Standard 100 µL
  Catalog Number MAK459D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge capable of RCF ≥ 10,000 × *g*
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2-8 °C.

Preparation Instructions

Bring all reagents to room temperature and shake or vortex briefly prior to assay. Briefly centrifuge small vials prior to opening.

SDS Standard: The solution can form precipitate when cold. To redissolve, gently heat the SDS Standard for several minutes and vortex to mix thoroughly.

Procedure

All samples and standards should be run in duplicate.

Notes:

(a) Bile Acids such as sodium deoxycholate interfere with this assay and cause high levels of cloudiness. They cannot be used in the assay.

(b) Samples should be clear and free of particles of turbidities. If Sample is colorless, no Sample Blank is needed.

Triton X-100 Standard Curve for use with Cationic or Non-Ionic Detergents

1. Prepare a 12 mM Triton X-100 Standard by mixing 45 µL of the 100 mM Triton X-100 Standard with 330 µL of purified water.
2. Prepare Triton X-100 standards in 1.5 mL microcentrifuge tubes according to Table 1. Standards may be frozen and re-used for future assays.

Table 1.
Preparation of Triton X-100 Standards

Well	12 mM Triton X-100 Standard	Purified Water	Triton X-100 (mM)
1	100 µL	-	12.0
2	80 µL	20 µL	9.6
3	60 µL	40 µL	7.2
4	40 µL	60 µL	4.8
5	30 µL	70 µL	3.6
6	20 µL	80 µL	2.4
7	10 µL	90 µL	1.2
8	-	100 µL	0

SDS Standard Curve for use with Anionic Detergents

1. Prepare a 25 mM SDS Standard by mixing 50 µL of the 250 mM SDS Standard with 450 µL of purified water.
2. Prepare SDS standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.
Preparation of SDS Standards

Well	25 mM SDS Standard	Purified Water	SDS (mM)
1	100 µL	-	25.0
2	90 µL	10 µL	22.5
3	80 µL	20 µL	20.0
4	70 µL	30 µL	17.5
5	60 µL	40 µL	15.0
6	50 µL	50 µL	12.5
7	40 µL	60 µL	10.0
8	-	100 µL	0

Standard Curve for Detergents Other Than Triton X-100 or SDS

Using pure stock of the detergent of interest, prepare a range of dilutions. Prepare at least eight dilutions. The lower detection limit of will be equal to the Critical Micellar Concentration (CMC) of the detergent. The remaining standard solutions must be above the CMC concentration in order to have sufficient data points for a standard curve. Include a "No Detergent" blank of purified water.

Assay Reaction

1. Transfer 20 µL of each Standard and Sample into separate wells of a clear flat-bottom 96-well plate.
2. If Sample is colored, transfer 20 µL of Sample into two separate wells for use as Sample and Sample Blank. Highly colored samples with an optical density above 0.1 at the wavelength of interest may require dilution before use in the assay.
3. Add 180 µL of Reagent A for Cationic/Nonionic Detergents (e.g., Triton X-100), or 180 µL of Reagent B for Anionic Detergents (e.g., SDS) to all Samples and Standards.
4. Add 180 µL of purified water to Sample Blank wells.
5. Tap plate to mix thoroughly.

Measurement

Read the optical density (OD) at 560 nm for Cationic/Nonionic Detergents or at 650 nm for Anionic Detergents.

Results

1. Subtract the No Detergent Standard OD value (Water in the Standard curve) from the remaining Standard OD values.
2. Plot the corrected OD values against the Standard concentrations.
3. If required, subtract the corresponding Sample Blank OD value from the Sample OD value.
4. Use the Standard curve to determine the Sample detergent concentration.
5. If the calculated detergent is above the highest Standard value, dilute the Sample in purified water and rerun the assay. Include the dilution factor in the calculation for detergent concentration.

Conversions:

1 mM Triton X-100 equals 0.0625% or 625 ppm.

1 mM SDS equals 0.0288% or 288 ppm.

Figure 1.
Typical Triton X-100 Standard Curve

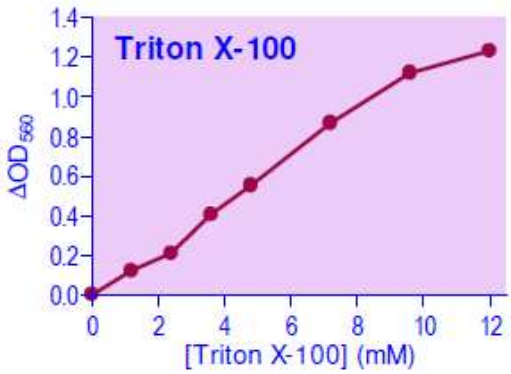


Figure 2.
Typical SDS Standard Curve

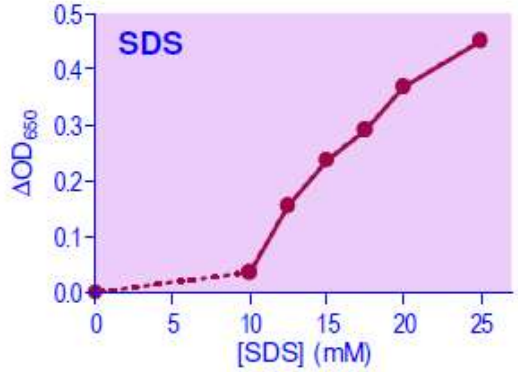


Figure 3.
Typical TWEEN 80 Standard Curve

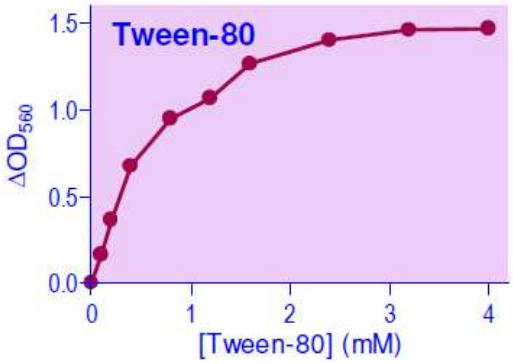


Figure 4.
Typical TWEEN 20 Standard Curve

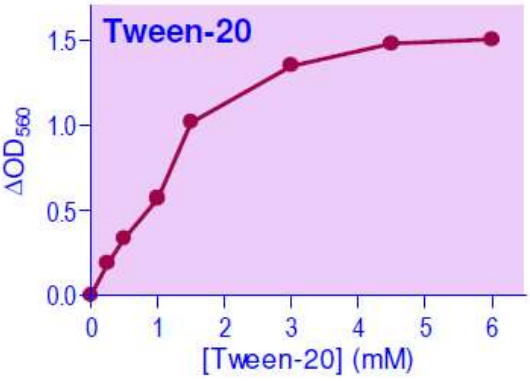


Figure 5.
Typical Brij 35 Standard Curve

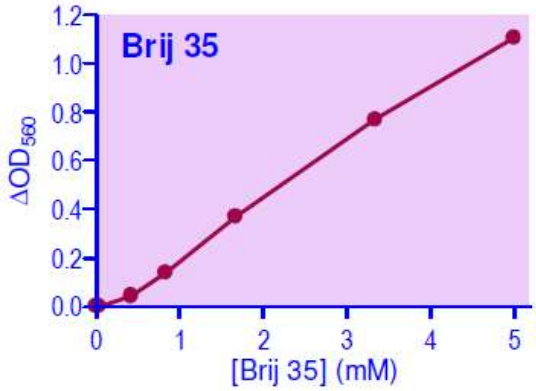
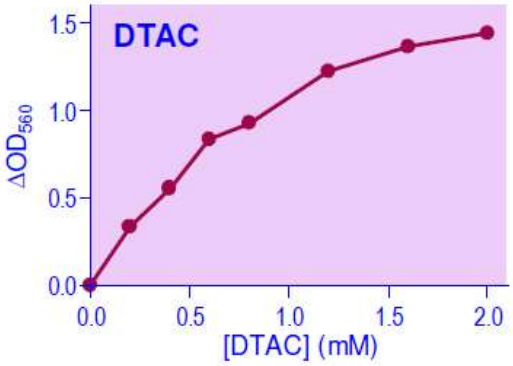


Figure 6.
Typical DTAC Standard Curve



References

1. Jing, J.L.J., et al., Hand sanitizers: A review on formulation aspects, adverse effects, and regulations. *Int. J. Environ. Res. Public Health*, **17**,3326 (2020).
2. Mukherjee, P., et al., Clouding behaviour in surfactant systems. *Adv. Colloid Interface Sci.*, **162**, 59-79 (2011).
3. David, V., Chapter 12 - Comments on Sample Preparation in Chromatography for Different Types of Materials. S. Moldoveneau (Author), Modern sample preparation for chromatography (pp. 411-446). Elsevier Science (2016).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.