QuantiChrom™ Detergent Assay Kit (DDTR-100)

Quantitative Colorimetric Detergent Determination at 560 nm and 650 nm

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-ready procedures for measuring detergent concentration in biological samples are very desirable. BioAssay Systems' detergent assay kit is designed to measure detergent directly without any pretreatment. Above the critical micellar concentration, detergent forms micelle in solution that trap the colorimetric dye. The intensity of the color is directly proportional to the detergent concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use 20 µL sample. Detection range: 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: 0.08 to 2 mM; SDS: 10 to 25 mM.
Simple and convenient. The procedure involves addition of a single reagent and measuring OD_{560nm} or OD_{650nm}.
Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Cuvette or 96-well plate assay.

APPLICATIONS

Protein Purification: Traces of detergent in purified protein samples.
Environment: Detergent determination in water and soil.

KIT CONTENTS (100 tests in 96-well plates)

For Cationic/Nonionic Detergents (e.g. Tween, Brij, Triton)
20 mL Reagent A, 100 µL 100mM Triton X-100 Standard

For Anionic Detergents (e.g. SDS)
20 mL Reagent B, 100 µL 250mM SDS Standard

Storage conditions. The kit is shipped at room temperature. Store contents at 4°C. Shelf life: 12 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Note: (*a*) Bile Acids like sodium deoxycholate interfere with this assay and cause high levels of cloudiness. They cannot be used in this assay. (*b*) Sample should be clear and free of particules of turbidities. If Sample is colorless, no Sample Blank is needed in Step 3. (*c*) SDS standard can form precipitate when cold. To remove, gently heat the standard for several minutes and vortex to mix thoroughly. (*d*). Bring the reagents to room temperature and shake briefly prior to assay.

1. Standard Curve. *Triton X-100 Standard Curve:* Dilute to 12 mM by mixing 45 µL 100 mM Triton X-100 Standard with 330 µL dH_2O. Dilute standard in dH_2O (*see Table*). Standards may be frozen and re-used for future assays.

   SDS Standard Curve: Dilute to 25 mM by mixing 50 µL 250 mM Standard with 450 µL dH_2O (*see Table*).
2. Transfer 20 µL diluted Standards and Sample into the wells of a clear flat-bottom plate. If Sample is colored, transfer 20 µL of Sample into two separate wells (for Sample and Sample Blank). Extremely highly colored samples with an OD 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 (Add 180 µL deionized water to Sample Blank wells). Tap plate to mix.
4. Read optical density at 560 nm for Reagent A or 650 nm for Reagent B.

Triton X-100 Standards Dilution Table

No	12 mM STD + H_2O	Vol (µL)	Triton X-100 (mM)
1	100 µL + 0 µL	100	12
2	80 µL + 20 µL	100	9.6
3	60 µL + 40 µL	100	7.2
4	40 µL + 60 µL	100	4.8
5	30 µL + 70 µL	100	3.6
6	20 µL + 80 µL	100	2.4
7	10 µL + 90 µL	100	1.2
8	0 µL +100 µL	100	0

SDS Standards Dilution Table

No	25 mM STD + H_2O	Vol (µL)	SDS (mM)
1	100 µL + 0 µL	100	25.0
2	90 µL + 10 µL	100	22.5
3	80 µL + 20 µL	100	20.0
4	70 µL + 30 µL	100	17.5
5	60 µL + 40 µL	100	15.0
6	50 µL + 50 µL	100	12.5
7	40 µL + 60 µL	100	10.0
8	0 µL + 100 µL	100	0

CALCULATION

Subtract blank OD (water) from the standard OD values and plot the OD against standard concentrations. Use the standard curve to determine the sample detergent concentration.

If the calculated detergent is above the standard curve, dilute the sample in dH_2O and rerun the assay.

Conversions: 1 mM Triton X-100 equals 0.0625% or 625 ppm. 1 mM SDS equals 0.0288% or 288 ppm.

CONSTRUCTING A STANDARD CURVE FOR OTHER DETERGENTS

1. Prepare a range of dilutions of your detergent of interest, preferably at least eight. The lower detection limit of all detergents will be equal to the Critical Micellar Concentration (CMC), so all standards should be above this concentration. Include a no detergent blank of dH2O.

2. Select the appropriate dye for your detergent. Cationic/nonionic detergents require Reagent A and anionic detergents require Reagent B.

3. Transfer 20 µL diluted Standards into separate wells of the plate.

4. Add 180 µL of appropriate reagent to all Standards. Tap plate to mix.

5. Read the optical density at 560 nm for Reagent A or 650 nm for Reagent B.

6. Subtract the optical density of the water blank from each standard, and plot the ΔOD against standard concentrations.

4. Connect the standard curve points. Detergent standard curves are often nonlinear, see the sample standard curves below.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pure stock of detergent of interest for standard curve, pipetting devices and accessories (e.g. 20 µL), centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760) and plate reader.

EXAMPLES

Samples that have been tested with this kit: Softsoap, Dawn dish detergent, Arm and Hammer clothing powder, White Blood Cell Lysing Buffers.

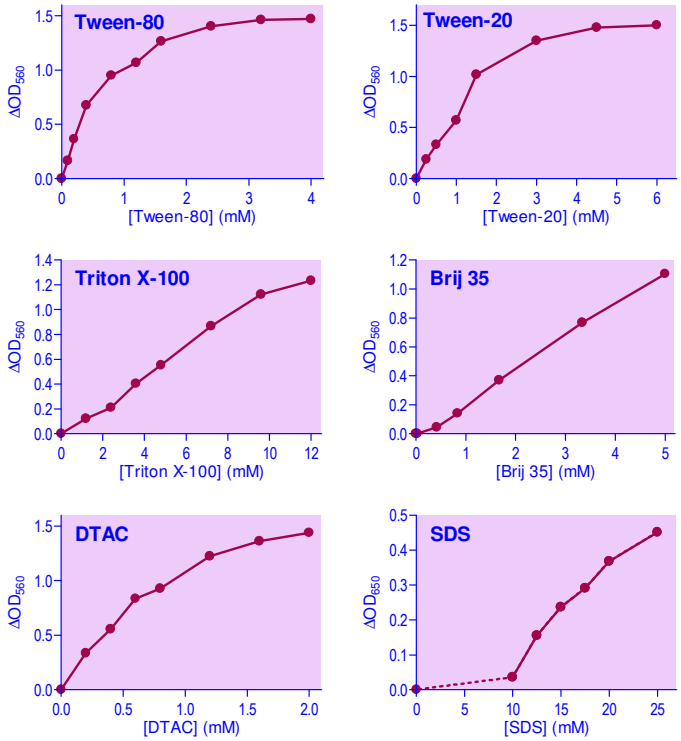
LITERATURE

1. Jing JLJ et al (2020). Hand Sanitizers: A Review on Formulation Aspects, Adverse Effects, and Regulations. Int J Environ Res Public Health. 17(9):3326.

2. Mukherjee P et al (2011) Clouding behaviour in surfactant systems. Adv Colloid Interface Sci.162(1-2):59-79.

3. David, V. (2016). 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.

SAMPLE DETERGENT STANDARD CURVES



Standard Curves in 96-Well Plate Assay