sageHLS™

HMW Library System

Quick Guide

E.coli cell suspension kit

Product:	**CEL-ECO1**
Revision A:	**March 27, 2017**

Reagents supplied by Sage Science:

1 ea. Wash Buffer, 25 ml; **E1**
1 ea. Spheroplast Buffer, 40 ml; **E2**
1 ea. Qubit Lysis Buffer, 25 ml; **E3**

Materials supplied or prepared by user:

Epicentre® Ready-Lyse™ Lysozyme Soln, 4 X 10^6 U: **Cat# R1804M**
NEB® Molecular Biology Grade BSA, 20mg/ml: **Cat# B9000S**
Qubit™ Fluorometer and HS DNA Assay kit
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)

Help: support@sagescience.com or call 978.922.1832

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A. Prepare Cell Culture

1. Users should prepare a fresh saturated overnight E.coli culture (grown with shaking on trypicase soy broth or LB broth).

B. Prepare Spheroplast Suspension

1. Place 500µl of the overnight culture into a 1.7ml microfuge tube.

2. Add 800µl of **Wash Buffer** (**E1**) to the culture in the microfuge tube.

3. Mix by inverting several times.

4. Pellet the cells in microcentrifuge at max speed (~14k x g) for 60 seconds.

5. Decant the supernatant and blot the tube on clean lab tissue.

6. Add 100µl of **Spheroplast Buffer** (**E2**) to the microfuge tube.

7. Prepare the Lysozyme Mix:
   a. Add 5µl of BSA to 1 ml of **Spheroplast Buffer** (**E2**)
   b. Add 2.5µl of Lysozyme to the Spheroplast Buffer/BSA mix
   c. Mix by gentle pipetting - take care to avoid generation of bubbles or foaming while mixing.

8. Add 1.5µl of Lysozyme Mix to the cell suspension.

9. Gently vortex.

10. Allow digestion for 30-40 minutes.

The digested cells may be stored at 4°C for up to 1 hour

C. DNA Quantitiation of Lysed Spheroplasts: Qubit Assay

1. Aliqout 5µl of the Spheroplast Suspension into a 1.7 ml microfuge tube.

2. Add 195µl Qubit Lysis Buffer (**E3**) to the microfuge tube.

3. Mix the spheroplasts by pipetting up and down to thoroughly mix.

4. Vigorously mix the lysed spheroplasts.

5. Add 600µl of TE buffer to the lysed spheroplasts.

6. Vigorously mix again.

7. Prepare the Qubit HS reagent according to the manufacturer's protocol.

8. Add 195µl Qubit HS reagent to three Qubit assay tubes.

9. Transfer 5µl of the lysed spheroplasts to each of the Qubit tubes:

10. Take a triplicate Qubit measurement of each tube

11. Calculate the amount of DNA in the lysed spheroplast sample:

Qubit reading in ng/ml x 0.200/5 (Qubit assay dilution factor) x 800/5 (Spheroplast lysis dilution factor) = ng/µl (DNA content of the Spheroplast Suspension)

D. Prepare Spheroplast Suspension for loading on the SageHLS

1. Dilute the Spheroplast Suspension with **Spheroplast Buffer** (**E2**) to prepare 10µg/70µl sample loads for the SageHLS.