SOP: SP049.1

LDH Assay

***Note: This protocol was created for Indo-Oceanic (IO) CFP QC. The intent of this protocol is to determine whether cell lysis has occurred in the IO CFP.**

Materials and Reagents:

1. Substrate Mix (Note 1)
2. Assay Buffer
3. LDH Positive Control (2µl Positive Control into 10ml PBS + 1%BSA,1:5,000 dilution)
4. Stop Solution
5. 96 well microtiter plate

Protocol:

1. _____ Add 50 µl of each sample to separate wells of the 96 well microtiter plate (note 2).

2. _____ Add 50 µl of the Positive Control to separate wells of the 96 well microtiter plate (note 2).

3. _____ Add 50 µl of the blank to separate wells of the 96 well microtiter plate (note 2). This should be the same buffer that the sample is in.

4. _____ Thaw the reconstituted substrate mix if applicable (Note 3&4).

5. _____ Add 50 µl of the reconstituted substrate mix to each well that contains sample, positive control, or blank.

6. _____ Wrap 96 well microtiter plate in foil and place in drawer to protect from light.

7. _____ Incubate at room temperature for 30 minutes.

8. _____ Add 50 µl of stop solution to each occupied well.

9. _____ Read the absorbance of the microtiter plate wells at 490 or 492nm using the Microplate reader within 1 hour after addition of stop solution. (Note 5)

10. ____ Print report

11. ____ Note the Log Mean (farthest column on right) for the sample of interest on report. If mean is $\geq$3.0, sample passes LDH assay; if <3.0, sample fails. (Note 6)

Notes:

1. To reconstitute substrate mix, add 12 ml of Assay Buffer to 1 vial of Substrate Mix. Reconstituted Substrate Mix may be stored for 6–8 weeks at –20°C without loss of activity.
2. Each positive control, sample, and blank should be assayed in triplicate.
3. Reconstituted substrate mix can be thawed in a room temperature water bath.
4. Keep sample out of direct light as much as possible
5. Use the "LDH Assay w Report" protocol for the Microplate reader.
6. "Passing" means there was minimal amount of LDH released indicating minimal cell lysis. "Failing" indicates the level of LDH present is indicative of cell lysis.

References:

Promega Corporation. http://www.promega.com/resources/protocols/technical-bulletins/0/cytotox-96-non-radioactive-cytotoxicity-assay-protocol/. Revised 8/12. Part#TB163.