Covaris

PROTOCOL

AFA-Liposome™ Formation: Phospholipon 90G M220 130µL

Summary of Operating Conditions

Table 1. Summary of Operating Conditions

AFA Instrument	**M220**
Peak Incident Power	40 W
Duty Factor	50%
Cycles per Burst	1000
Duration	600 seconds
Bath Temperature	20°C
Power Mode	frequency sweeping
Degassing Mode	N/A
Volume	130µL

Recommended settings are subject to change without notice.

See http://covarisinc.com/resources/protocols/ for updates to this document.

Supplies

Table 2. Equipment List

Item	**Description**	**Part Number**
Sample Vessel	microTUBE Screw Cap 6x16mm	520146
Sample Holder	Holder for 520146	500358
Focused-ultrasonicator	M220	500295
Sample	Phospholipon 90G	Lipoid
Buffer	0.1 M PBS	Sigma

Operating Conditions

1. Turn on the M220 instrument and the computer, double click the SonoLab icon, fill the wash bottle with distilled or deionized water, and insert the *tube holder* .
2. Fill the water bath, place the sample tube in the holder. Place in the instrument.
3. Load the *Sliding Weight* on the top of the tube and close the *safety cover*; Create/Load a method then click "***Run***" to start a process; Click "***Pause***" to pause the process, or click "***Stop***" to terminate the process;

4. Remove *Tube Holder Insert* and drain the water; Wipe the surface of the water bath with lint-free swab; Air dry for next use or shut down before idling for long period of time.

Recommendations Specific for AFA-Liposome™ Formation

The Covaris AFA process is highly reproducible; however steps should be taken to ensure the best results. The bath water is employed to couple acoustic energy to the sample vessel, thus attention must be paid to the following water treatment attributes to obtain the best results:

1. *Purity*: When applying acoustics in rate-limited applications, foreign materials such as algae and particulates may scatter the high frequency focused acoustic beam. Bath water should be pure distilled or DI water, changed daily or cleansed by a Covaris Water Conditioning System.
2. *Temperature*: Warmer temperatures promote less forceful collapse of acoustic cavities within the sample fluid. Bath temperature (as reported by SonoLAB software) should therefore be closely controlled and matched run-to-run and day-to-day. Employ the temperature alert feature in SonoLAB to warn of a failure to maintain control of bath temperature**.**
3. *Level*: Attention should be paid to maintaining a consistent water level, according to published protocols. If using a Covaris Water Conditioning System, check levels daily to restore water lost to evaporation.
4. ***Trapped air pockets:*** **Air trapped in the vessel provides a reflective surface for the acoustical energy. When processing begins, any air pockets disperse as a multitude of small bubbles. These bubbles deflect and scatter the acoustic waveforms, effectively defocusing the energy and reducing overall effectiveness. For maximum performance, it is imperative that vessels be loaded full with aqueous sample, and air pockets minimized.**

In summary, when employing the Covaris AFA, control and verification of treatment attributes and water quality will reduce variance and promote consistent, satisfactory results.

Method

1. Set up the Covaris M220 at the appropriate temperature following the operating conditions above.
2. Weigh out 2.6 mg of Phospholipon90G and add the sample to the sample vessel.
3. Fill the tube with buffer (approximately 130 µL), and cap the tube. Be careful not to introduce a bubble into the bottom of the tube. This may happen if the water is added too quickly.

   CAUTION: The bottom of the tube is in the acoustic field. Therefore, a bubble in the sample will deflect energy and induce variable results.
4. Carefully load the sample vessel into the appropriate holder, and place in instrument.
5. Initiate and Run process according to the operating conditions specified in Table 1.

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Supplementary Data

Description

A Malvern Zetasizer Nano ZS-90 may be used to analyze the AFA-Liposomes™ formed using the Covaris M220. The sample prepared above will be utilized for analysis.

Supplies

Table 3. Supplementary Equipment List

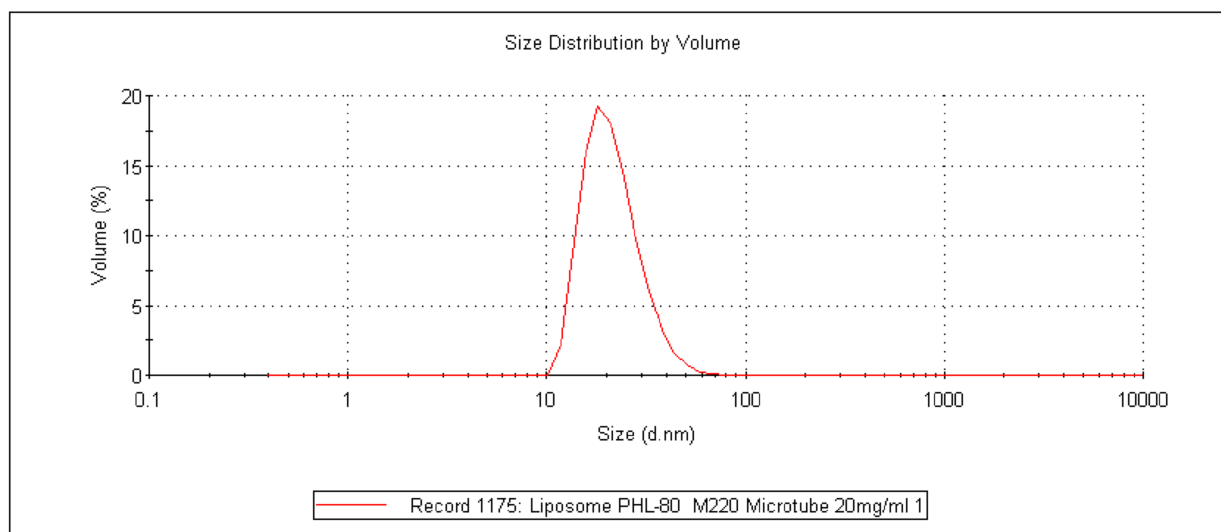
Item	Part Number
Malvern Zetasizer Nano ZS-90	ZEN3690
Sample Cuvettes	DTS0012

Method

1. Set up the Malvern Zetasizer according to its setup instructions.
2. Add 1 mL of deionized water to the cuvette.
3. Aliquot 130µL of prepared sample into cuvette. The prepared sample is the sample prepared on the previous page after it has been treated with AFA. Cap the cuvette.
4. Shake the cuvette by hand until the sample is dispersed evenly in the cuvette without air bubbles.
5. Place in Zetasizer instrument and run Volume Distribution Analysis.

Typical Output Readings: Volume

Figure 1. Size Distribution by Volume



Typical Output Readings: Variation

Table 4. Variation in Results

Process	Z-average (d.nm)	PdI
1	27.3	0.130
2	25.7	0.080
3	25.7	0.086
4	26.3	0.086
5	26.4	0.117
Average	26.3	0.099
SD	0.56	0.0199
CV	2%	20%

Figure 2. Variation in AFA-Liposome™ Formation Results