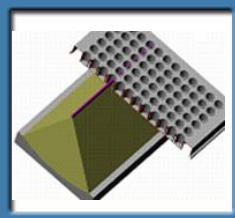
Benefits of Covaris AFA Ultra Sonication on the HTS Process from Compound Master to HTS Assay Plate

[1]Susan Kirby, [1]Phil Robinson, [1]Jon Curtis, [2]Jim Laugharn, [3]Catherine Wark

[1]KBiosciences, Hoddesdon, Hertfordshire, UK

[2]Covaris, Woburn, Massachusetts, USA

[3]BMG LABTECH, Aylesbury, Bucks, UK

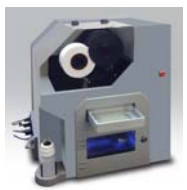


Introduction

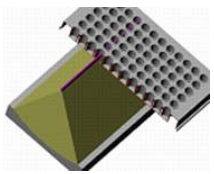
The Covaris Process, termed Adaptive Focused Acoustics (AFA), uses high frequency non contact ultra sonics derived from medical diagnostic ultrasound imaging and therapeutic lithotripsy applications, such as kidney stone disruption.

By applying this technology Covaris have developed a fully linear AFA generating transducer capable of automated high speed mixing of both CM and HTS plates, independent of density.

An evaluation was carried out to determine the effectiveness and utility of the linear AFA transducer in the Drug discovery process, at both the CM master plate and HTS assay plate stages.



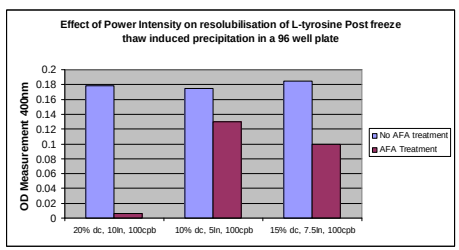
L800 AFA mixer



AFA transducer

Evaluation

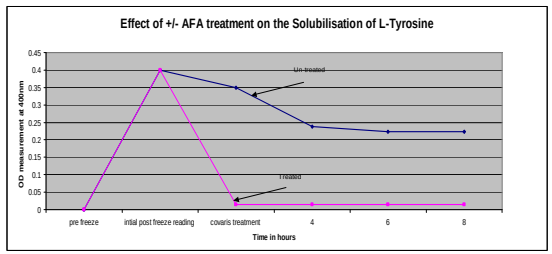
A model system was chosen to mimic the actual process of preparation of HTS assay plates from 384 well master plates. L-tyrosine is a poorly soluble amino acid with a molecular weight in the range of modern day chemical libraries. It is known that upon freeze thawing a solution that precipitation will occur. A solution of L-tyrosine at 22mg/ml was made and dispensed to a 96 well Greiner polypropylene plate. The plate was frozen at –20ºC overnight and removed from the freezer and allowed to thaw. Readings for Optical Density at 400nm were taken after differing power levels were applied to the plate via the linear Covaris AFA transducer. It was unknown at which AFA conditions to treat to achieve re-solubilisation. The plate was then reread at varying times. The linear transducer was moved for single pass at 3mm/sec.



Key : dc = Duty cycle, I = Intensity, cpb = cycles per burst

To determine the effectiveness of these optimised conditions for the higher density 384 well plate a further experiment to assess the performance of AFA was carried out.

A 384 well Greiner plate containing 50ul was subjected to AFA treatment at 20% dc, 10I, 100cpb, with a Z height of 15mm. The linear transducer was passed under the plate at 3mm/sec for one pass only, taking ~ 90secs per plate.



Identical data was demonstrated for volume ranges from 10ul to 50ul.

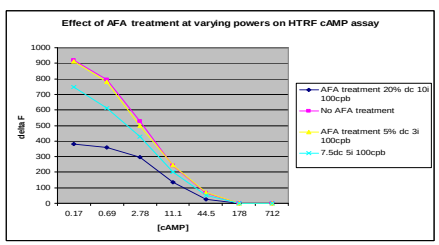
Assessment of AFA mixing at the HTS assay stage

Having demonstrated the effectiveness of AFA generated via a Linear transducer in CM, we undertook a further study to evaluate its performance in two different HTRF assays. The assays used were cAMP and TNF-alpha from Cisbio. Both assays proved to be highly reliable, easy to use tests for the evaluation. Assays were performed in black Greiner low volume 384 well plates. The results & data analysis were read on the BMG Pherastar instrument, chosen because of its high degree of accuracy and reproducibility.

The two chosen assays differ in their type (competitive & direct) and speed of developing. This served as a good indicator of assay type for the evaluation.

cAMP Assay

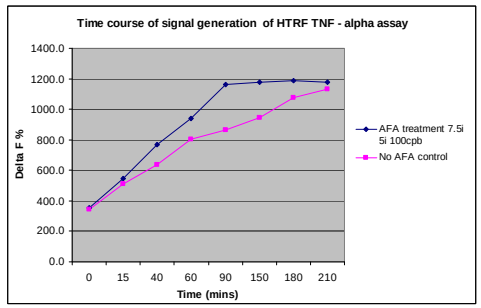
The cAMP assay from Cisbio is a competitive assay that develops very fast (<15 mins). The assay components were combined into a 20ul volume and read after 15mins. The AFA treated plate was treated under high power as defined by the work on the resolubilisation of L-Tyrosine, as well as at lower power levels.



The data shows that treatment of HTS cAMP assay plates at high power levels can have a detrimental effect on the data quality.

TNF-alpha Assay

The TNF-alpha assay develops at a slower rate than the cAMP assay. An experiment applying AFA energy at levels similar to the cAMP experiment was conducted to ascertain the effectiveness in the slower TNF-alpha assay.



The data shows that treatment of the HTS TNF-alpha assay plates at varying power levels can have a positive effect on the speed of the development of the assay, this is especially clear when comparing the 90 mins vs 210 mins with and without AFA treatment.

Conclusion / Discussion

Re-solubilisation of compounds in High–Density storage formats is possible with the linear AFA transducer at high power.

Each individual assay needs to be optimised for AFA treatment conditions

Re-solubilisation of compounds is not possible without splashing – thus requiring plate sealing and centrifugation post treatment.

AFA treatment of the compound under test in assay medium (aqueous environment) requires high power treatment and thus should not contain the biological assay components.

A pre-dilution of the compound into assay medium with an AFA re-solubilisation step is therefore recommended.

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