Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

HY-LiTE® Testing of Paints and Coatings Samples.

Regular monitoring of the microbiological activity in paints and coatings is an important tool to ensure timely action (such as treatment with Biocide), thereby reducing quality and process problems and potential line down-time.

Materials:

HY-LiTE® Plus ATP pens (1.30895.0021)

HY-LiTE® Free ATP pens (1.30194.0021) – Optional

Equipment:

HY-LiTE® 2 Luminometer (1.30100.0001)

Method, Liquid Samples:

Unless it is already validated that a different sample dilution rate is required, it is recommended to use a 1:3 sample dilution.

For routine monitoring purposes, it is usually sufficient to measure Total ATP only using the HY-LiTE® Plus pen.

In certain circumstances (e.g. in samples containing high levels of solvents or shortly after dosing with cell-lysing biocide), it may be beneficial to measure both extra-cellular ("Free") and Total ATP.

Both tests can be performed on the same sample, provided the sample is always sampled with the Free ATP pen (red cap) BEFORE it is sampled with the HY-LiTE® Plus pen (green cap).

Note: Once the Total ATP pen has been dipped in a sample, the lysing agent on the sampling stick will release the cellular ATP from all cells in the sample, and it can no longer be used for measuring extra-cellular (Free) ATP.

If performing sample dilution, follow the directions in the pack insert.

After mixing of the diluted sample, dip the sampling stick of a Free ATP pen (red cap) in the dilution tube (while this is still attached to the HY-LiTE® Plus pen). Press the stick into the cuvette, press and turn the top to release the reagent, shake to mix the reagent and insert the pen into the Luminometer.

Record the result.

Once the Free ATP measurement has been performed, close the dilution tube and separate it from the Plus pen. Sample with the HY-LiTE® Plus (green cap) sampling pen, then press the stick into the cuvette, and process and read as above.

Results:

If samples are always diluted 1:3, you can choose to set the action limits based on direct measurement of a 1:3 dilution.

If results of diluted and un-diluted samples are to be compared, results of diluted samples should be multiplied by the dilution factor before interpretation.

Dilution rate	Sample volume added	Direct reading (RLU)	Multiplication factor	Corrected result
1:3	0.5 ml	x	3	3*x
1:5	0.25 ml	y	5	5*y

Interpretation:

If no previous experience exists, it is recommended to set limits based on at least 50 individual measurements obtained over a period of time.

Warning and action limits can be set either based on using non-parametric distribution statistics, by calculating the RLU equivalent to the desired percentile (e.g. 90% percentile = Warning and 99% percentile = Action or 75% percentile = Warning and 95% percentile = Action).

Alternatively perform log-transformation of all results (as the results are most likely following a log-normal distribution) and then apply standard Statistical Process Control to the data (X-bar, R chart).

Results from sampling of surfaces will most likely require different interpretation limits to those of liquid bath samples.

The results of Free and Total ATP can be compared by calculating the ratio (or percentage) of free to total ATP (RLUFree/RLUTotal).

Under normal circumstances this will be less than 10%, however, in systems (e.g. coating baths) with low turnover, the proportion of free ATP may increase, in which case, the action limits applied may need to be revised.

Free ATP is indicative of Biomass which is no longer alive, and which may therefore be harmless. However, accumulation of Free (extra-cellular) ATP is also indicative of presence of extra-cellular enzymes and metabolites, so could still be indicative of problems.

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