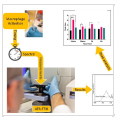

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ATR FTIR Spectoscopy of Aqueous Cell Cultures V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bdidi4a6

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ABSTRACT

ATR-FTIR Spectroscopy of aqueous cell culture samples is explained step-by-step. Infrared spectra acquisition, processing and analysis is included briefly.

Spectra acquisition

1 Arrange the temperature of the working environment to **25 °C**

2 Clean the ATR crystal using a cellulose wipe and water-ethanol-water sequence.
Scratching the crystal surface must be avoided.

ATR FTIR
Spectroscope
Perkin Elmer Inc., Norwalk, CT, USA X

3 Leave the crystal to air-dry

4 Select “Scan and Instrument Setup” and input sample name, scan range (650-4000 cm^{-1}), scan number (128), and spectral resolution (2 cm^{-1}). This step needs optimization for specific sample being used.

Spectrum One
by Perkin Elmer

5 Scan the backgroung spectrum to overcome the probable atmospheric interference that can be triggered by H_2O and CO_2 molecules present in the air, subtracted automatically by the software.

6 Additionally, record PBS as sample (subtract this manually from each spectrum)
This eliminates interference of PBS and flattens the water band located around 2125 cm^{-1}

7 Put **1 µl** cell samples and let to dry with a mild N_2 flux for **00:05:00** . This allows cells to settle on the crystal. 5m

7m

8 Scan the sample. Process takes about **00:07:00** to finish.
As the scanning process goes on, one must be sure that the quality check sign is everytime green.

9 Scan each sample under the same conditions in three independent scans (the average constitutes a replicate n). Replicates (n=5) are used in data analysis.
This minimizes intra-sample variability and eliminates possible variation that might arise from experimental conditions.

Spectral pre-processing

10 The avarage of triplicates is taken manually.

Spectrum One
by Perkin Elmer

11 13-point Savitzky-Golay smoothing

12 Baseline correction at 3800-2750-1800-900 cm^{-1}points.

Principal Component Analysis (PCA)

13 Import spectra as data file into

Unscrambler 11.0
by Camo, NO

14 Define the data sets to 3000-650 cm^{-1}region.

15 Make the PCA Model.
Define the regions with high variation using the Loadings plots.
Check how the replicates are grouping and if there are outliers in Scores plots.

Spectral Calculations

16 Peak labelling can be done automaticaly. For increased precision we measured manually:
Measure the center of weight of each band. Read the frequency.
Compare the peaks you obtained, to the literature:

Zanyar Movasaghi, Shazza Rehman, and Ihtesham ur Rehman. Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. Applied Spectroscopy Reviews.
http://10.1080/05704920701829043

Make an excel file of your peaks and their assignment to the functional groups.
For side-peak band assignment, the second derivative spectrum is used.

17 Concentration studies are carried out by integrating the curves under the peaks.

18 For conformational alteration studies, bandwidth measurements are done manually using the 75% height of the peaks.