

Cyanobacterial Pre-Fractionated Extracts

96 well microtiter plates

Introduction

Cyanobacteria are well known to produce unique, structurally diverse, and potentially bioactive secondary metabolites.¹⁻⁴ Despite the fact that their potential for natural product production nowadays is acknowledged, they are still an untapped source. This results in a remarkably low rediscovery rate and makes cyanobacteria an attractive source for novel secondary metabolites.

Cyano Biotech has screened its extensive cyanobacteria strain collection using HPLC/MS/DAD/ELSD. We have selected productive strains for upscaling to generate cyanobacteria biomass and medium extracts. These extracts have been fractionated by centrifugal partition chromatography (CPC) to yield about 20 qualified fractions per extract. To reduce unnecessary replication of compounds in fractions, chemically similar fractions resulting from the CPC fractionation have been pooled prior to preparation of the final fractions. While this results in a varying number of fractions per strain, it ensures that no unnecessary screening assays need to be performed. Each final fraction has been analysed by HPLC/DAD/HRMS² and arrayed in equal amounts of 25 µg per well within microtiter plates, resulting in a high quality focused screening library of cyanobacterial metabolites.

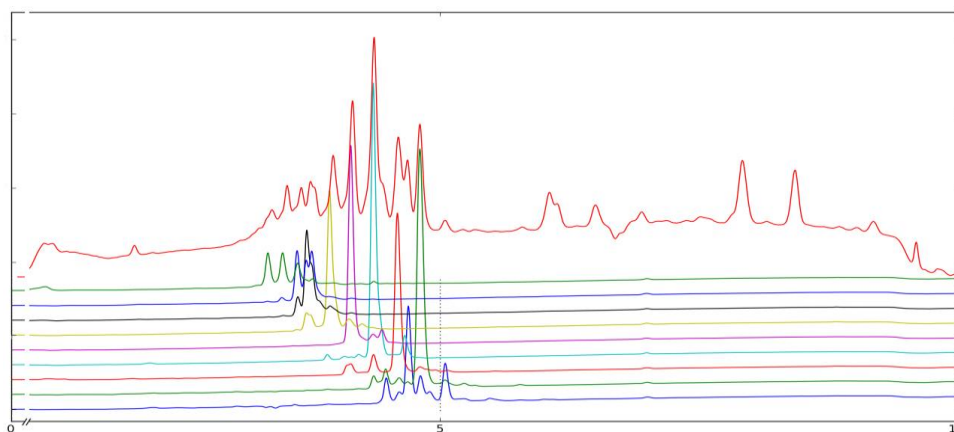
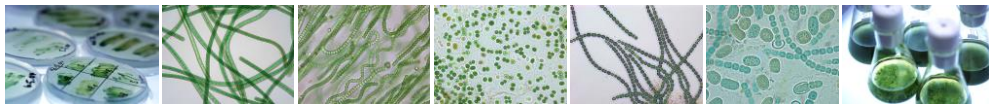
Features of the library

- Hand-picked cyanobacteria strains specially selected for a large number of chemically diverse compounds; strains are cryopreserved and sufficient biomass has been harvested to ensure immediate resupply
- Gentle fractionation of extracts using liquid-liquid chromatography
- Each well of the sealed polypropylene 96-well microtiter plates contains approx. 25 µg of dry fraction
- HPLC/DAD/HRMS² data available for all fractions, ensuring rapid follow up of hits

The library of pre-fractionated extracts is available in two Sets:

- The **Complete Diversity Set** contains all compounds present in the cyanobacteria extracts, including minor compounds. This library ensures complete coverage of the cyanobacteria extract richness, resulting in a higher number of microplate wells per strain. This library is most suitable if customer resources allow the screening of a larger number of fractions and customers are willing to follow up also on minor compounds.
- The **Major Compound Set** contains selected fractions of the Complete Diversity Set. Due to the refined nature of this library set, the fractions are less complex (maximum number of major compounds in fraction is 5), with many fractions containing only one single compound with > 80% purity (see figure below). This library is most suitable for customers with restricted screening resources wishing to assay only major cyanobacterial compounds in less complex fractions. Follow-up of hits from this library is more convenient than from the Complete Diversity Set, but potentially highly active minor compounds will be excluded from the screening.

Furthermore, customized libraries can be provided (e.g. focused screening of specific genera; specific amount per fraction or specific plate layout).



Overlaid HPLC/UV chromatograms of the Major Compound Set fractions from a *Nostoc* strain; crude extract is shown as upmost trace.

Storage and use of the library

The microtiter plates should be stored at or below 4°C until reconstituted. Reconstitution of the fractions in the wells should be performed either in neat DMSO or in a DMSO/water/buffer solution as appropriate.

If reconstituted in DMSO: Store below -20 C. Use within 1 year. Repeated freeze-thawing might result in precipitation of some material and should be avoided if possible.

If reconstituted in DMSO/water or DMSO/buffer: Store at 4°C. Use within 2 weeks.

Cyanobacterial secondary metabolites have an average weight of about 650 g/mol. It might be appropriate to consider the average weight when calculating the final assay concentration.

Hit follow-up

Following screening of the library the customer should contact Cyano Biotech to follow up hit fractions. This can include dereplication of known compounds, subfractionation of the fractions, isolation and structure elucidation of active compounds, compound diversification and optimization, strain development towards the customer's specific needs, and resupply of compounds. These services are provided in close coordination with the customer.

Safety

This product contains cell free fractions from cyanobacteria strains isolated from diverse environments and localities. The samples contain uncharacterized chemicals and therefore should be treated as potentially hazardous.

Always wear personal protective equipment (laboratory gown, safety glasses, and gloves) and dispose unused material as laboratory waste.

- [1] Timo Niedermeyer and Mark Brönstrup. 2012. Natural product drug discovery from microalgae. In: *Microalgal Biotechnology: Integration and Economy*, ed. Clemens Posten and Christian Walter, 169-202. De Gruyter.
- [2] Rahul Kunwar Singh, Shree Prakash Tiwari, Ashwani K Rai, and Tribhuban M Mohapatra. 2011. Cyanobacteria: an emerging source for drug discovery. *The Journal of Antibiotics* 64: 401-412.
- [3] Kevin Tidgewell, Benjamin R Clark, and William H Gerwick. 2010. The Natural Products Chemistry of Cyanobacteria. In: *Comprehensive Natural Products II: Chemistry and Biology*, ed. Lewis Mander and Hung-Wen Liu, 141-188. Elsevier.
- [4] Heike Sielaff, Guntram Christiansen, and Torsten Schwecke. 2006. Natural products from cyanobacteria: Exploiting a new source for drug discovery. *IDrugs* 9: 119-127.