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Product Information

Short Chain Fatty Acids Kit

Catalog Number **SBR00030**
Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

Short chain fatty acids (SCFAs) are saturated fatty acids with less than 6 carbons. SCFAs are produced in the gut by microbial fermentation of dietary fibers.[1] The most important and researched SCFAs are Acetate (Ac), propionate (Pr) and butyrate (Bu).[2] SCFAs are predominantly found in the colon and cross the intestinal epithelium to the blood stream. SCFAs were found to be beneficial and effective in prevention of obesity, non-alcoholic fatty liver disease and insulin resistance.[3,4]

SCFAs can serve in various applications including *in vitro* and *in vivo* administration to study the effect on immune system activation,[5] protect epithelial cells from LPS penetration by tight junction formation induction,[6] effect insulin secretion,[7] and more. In these studies, SCFAs were administrated at the following concentration range:

In vitro: Acetate 1-10mM, propionate and butyrate 0.1-1 mM

In vivo: Acetate, 70 mM, propionate, 30 mM, butyrate, 20 mM.

The Short Chain Fatty Acid kit includes ready-made aqueous solutions which have been 0.2 µM filtered and are tested for endotoxin. These can be easily administrated for various experiments.

In addition, a mixture of Sodium Acetate, Sodium Propionate, and Sodium Butyrate at 0.1 M is provided to assist in calibration of downstream detection of SCFAs by HPLC, GC-MS, or LC-MS.

Components

Sodium acetate 1 M Catalog Number SBR00030A	100 mL
Sodium propionate 0.5 M Catalog Number SBR00030B	100 mL
Sodium butyrate 0.5 M Catalog Number SBR00030C	100 mL
Short chain fatty acids mix 0.1 M mix of Sodium Acetate, Sodium Propionate and Sodium Butyrate, Catalog Number SBR00030D	1 mL

Reagents and Equipment Required but Not Provided.

- 2,3,4,5,6-Pentafluorobenzyl bromide (PFB-Br): Catalog Number 90257 or equivalent
- Nukol™ Capillary GC Column, size × I.D. 30 m × 0.25 mm, d_f 0.25 µm, Catalog Number 24107
- Ascentis® Express C18, 2.7 µm HPLC Column 2.7 µm particle size, L × I.D. 10 cm × 4.6 mm, Catalog Number 53827-U
- Ascentis Express C18, 2.7 Micron Guard Cartridge, 2.7 µm particle size, L × I.D. 5 mm × 4.6 mm, Catalog Number 53508-U
- Ascentis Express Guard Cartridge Holder, Catalog Number 53500-U
- Acetone, Catalog Number 650501 or equivalent
- Sulfuric acid (H_2SO_4), Catalog Number 339741 or equivalent
- Diethyl ether, Catalog Number 309966 or equivalent

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped at ambient temperature. For long-term storage, store at –20 °C.

Procedure
HPLC, LC-MS and GC-MS can be used to test the mount of SCFAs in biological samples.

Detection by LC-MS/HPLC
When analyzing by LC-MS, a derivatization should take place. 2,3,4,5,6-Pentafluorobenzyl bromide (PFB-Br) is recommended for derivatization of SCFAs in an aqueous acetone mixture.

1. Prepare derivatization reactions for each component as shown in Table 1.

Table 1. Derivatization reaction mixtures

Component	SCFA	Acetone	PFB-Br
Acetate (Sodium acetate 1 M)	100 µL	900 µL	15 µL
Propionate (Sodium propionate 0.5 M)	200 µL	800 µL	15 µL
Butyrate (Sodium butyrate 0.5 M)	200 µL	800 µL	15 µL
Short chain fatty acids mix	100 µL	900 µL	3 µL

2. Mix to homogeneous mixture according to quantities in the above table in screw cap glass vial.
3. Close cap and heat the solution to 65 °C for 18-24 hours for complete reaction (See Figure1 for example

LC Conditions:
Column: Ascentis® Express C18, 2.7 µm HPLC Column 10 cm × 4.6 mm (Catalog Number 53827-U)
Column guard cartridge: Ascentis Express C18, 2.7 Micron Guard Cartridge, 5 mm × 4.6 mm (Catalog Number 53508-U)
Column guard holder: Ascentis Express Guard Cartridge Holder (Catalog Number 53500-U)
Column oven temperature: 25 ºC
Gradient: See Table 2
Flow rate: 1 mL/min
Detection: At 210 nm and 260 nm
Injection volume: 5 µL

Table 2. Recommended solvent gradient for LC-MS/HPLC analysis

Time [min]	Acetonitrile [%]	Water + 0.1% TFA [%]
0	20	80
15	80	20
18	20	80
20	20	80

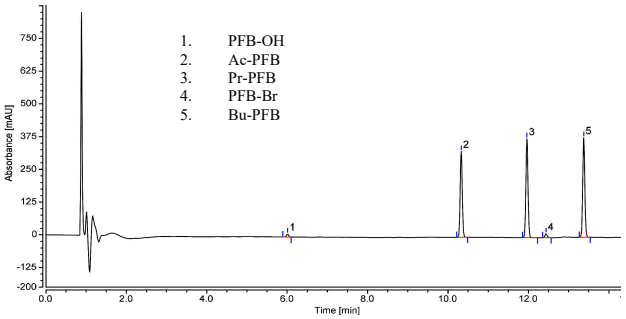

Figure 1. HPLC chromatogram of the SCFA mix at 210 nm. The Short chain fatty acids mix was derivatized and analyzed according to the instructions.

Calibration Curve Generation

Both single SCFAs and the SCFA mix can be used to form calibration curves.

1. Derivatize the SCFA based on the Derivatization instructions under Detection by LC-MS/HPLC.
2. Dilute the derivatized SCFA mixture to several concentrations and analyze by HPLC following LC Conditions instructions.
3. Collect peak area information for each concentration and plot a calibration curve as shown for the example below (see Figure 2).

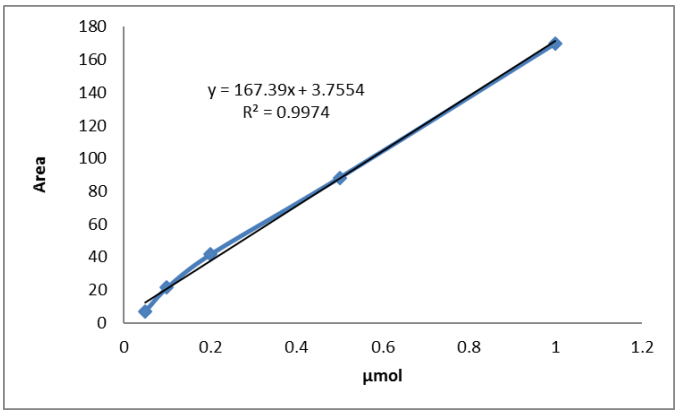

Figure 2. Example of calibration curve for propionate generated using Sodium propionate 0.5 M.

Detection by GC-MS

For GC-MS analysis Sigma-Aldrich recommends using a Nukol Capillary GC Column, size × I.D.
30 m × 0.25 mm, d_f 0.25 µm, Catalog Number 24107

GC conditions:
Oven: 185 ºC
Carrier gas: Helium 20 cm/sec
Injection volume: 1 µL

Analysis of SCFAs mix and single SCFA by GC-MS

1. Use a glass vial for the extraction of the short chain fatty acid from the aqueous solvent.
2. Add to the glass vial 100 µL from Short chain fatty acids mix or a single SCFA solution. Add 900 µL diethyl ether. Mix well.
3. Add 5-10 µL concentrated sulfuric acid (H_2SO_4).
4. Collect the upper ethereal phase which contains the SCFA(s). Avoid taking the aqueous phase.
5. Analyze by GC-MS according to instructions above. Example of results for the mix analysis shown in Figure 3.

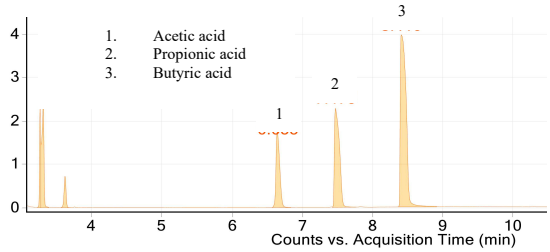

Figure 3. GC chromatogram of the SCFA mix. The Short chain fatty acids mix was extracted and analyzed according to the instructions.

Detection of SCFA in Biological Samples

The LC method described above can be utilized to detect SCFAs in plasma and feces. Sample processing procedures may vary.

The following is an example of detection of propionate in plasma using a calibration curve generated with Sodium propionate 0.5 M.

1. Sample preparation. Add 800 µL acetone to 200 µL plasma in a 1.5 ml tube. A white precipitate may form.
2. Centrifuge the sample mixture with precipitate from Step 1 for 2 minutes at 14,000 RPM.
3. Transfer the clear supernatant to a clean screw-cap glass vial.
4. Add 3 µL PFB-Br and cap the vial. Heat the solution to 65 °C for 18-24 hr for complete reaction.
5. After incubation, analyze the derivatized sample using LC-MS by the method provided above (see Figure 4).

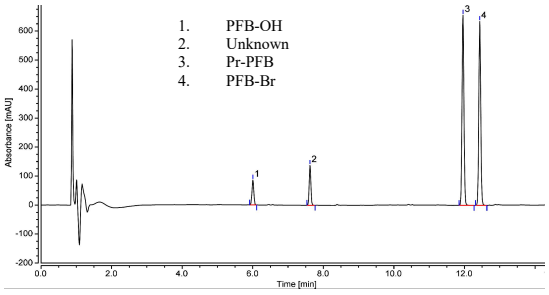

Figure 4. Example of HPLC spectrum of derivatized sample extracted from plasma and analyzed at 210 nm. Using a generated standard calibration curve, the propionate amount of plasma sample was found to be 0.88 µmol.

References

1. Koh, A., et al., From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*, **165(6),** 1332-1345 (2016).
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7. Pingitore, A., et al., The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. *Diabetes Obes. Metab*., **19(2),** 257-265 (2017).

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