

# Agilent AdvanceBio workflows for spent media analysis





# Achieve fast, reproducible analysis of bioreactor cell culture media

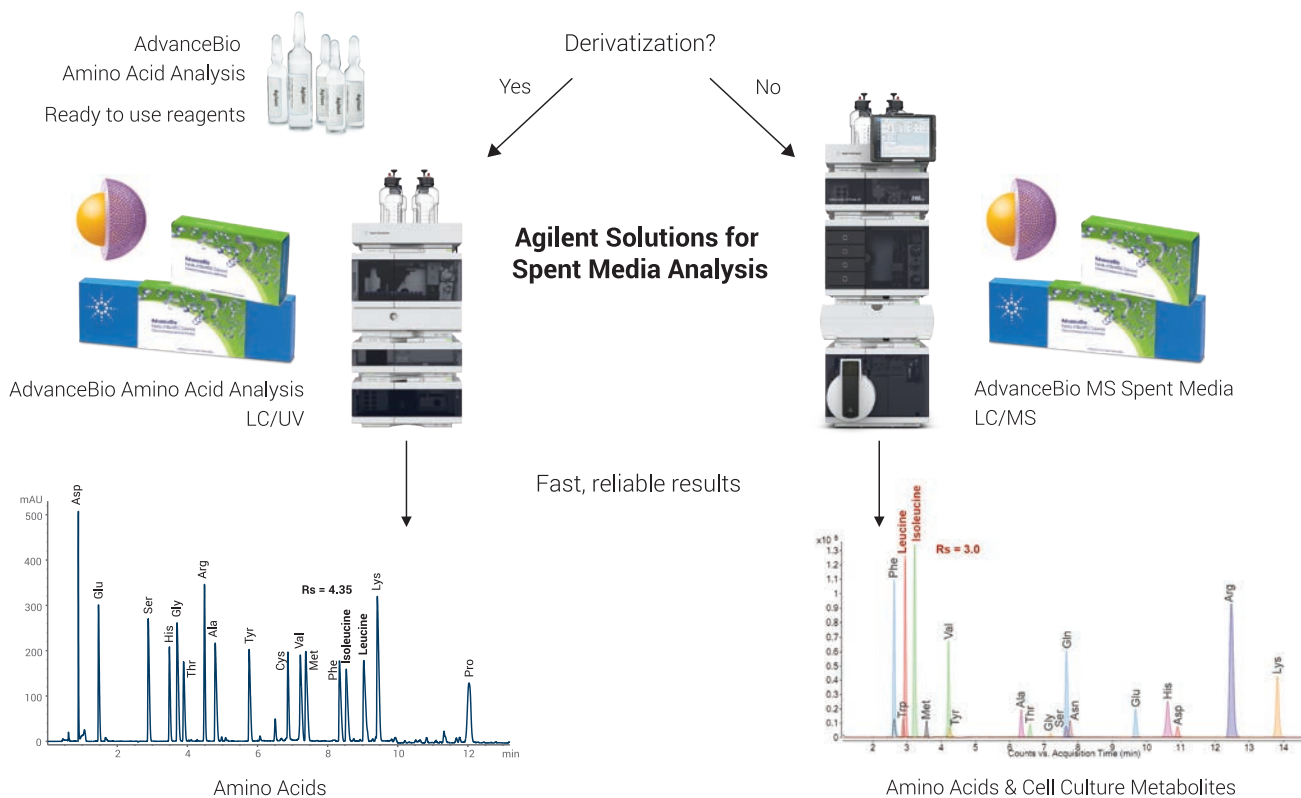
AdvanceBio columns from Agilent make it easier for your biotech lab to analyze amino acids and other small metabolites in spent cell culture media—with or without sample derivatization. Columns for both solutions are tested with amino acids to ensure quality and performance. Simply choose the workflow that suits your needs.

## Choose the Agilent AdvanceBio Amino Acid Analysis kit for industry-standard LC/UV analysis

- Get automated online derivatization of amino acids with reverse-phase LC separation and UV detection.
- Use any Agilent LC system.
- Minimize your investment in instrumentation and expertise.

## Choose Agilent AdvanceBio MS Spent Media columns for fast, underivatized LC/MS analysis

- Analyze amino acids and other cell culture metabolites with a single method: HILIC LC separation with MS detection.
- Eliminate the need to derivatize your sample.
- Use any LC/MS system.
- No need for baseline chromatographic resolution with MS detection.



# Analyzing derivatized amino acids via LC/UV



The AdvanceBio Amino Acid Analysis kit makes derivatization simple, using reagents conveniently packaged under a single part number and automated derivatization in the autosampler. Complete method details can be found in our how-to guide to amino acid analysis (publication number 5991-7694EN).

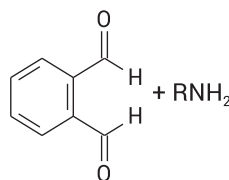
**Dependable:** OPA and FMOC derivatization chemistries are well established.

**Economical:** High-pH resistant, chemically modified silica extend the life of the columns.

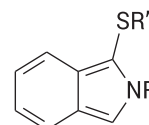
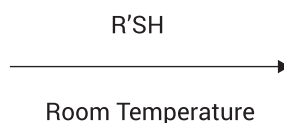
**Flexible:** Compatible with both HPLC and UHPLC systems via 2.7  $\mu\text{m}$  Poroshell particles.

Sample derivatization is fully automated in the autosampler of the Agilent LC stack, eliminating the time, errors, and variability associated with manual sample preparation.

## Ortho Phthalaldehyde (OPA)

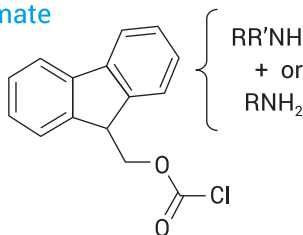


Non-fluorescent  
Does not absorb at 338 nm

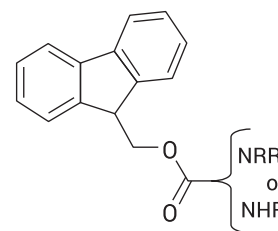
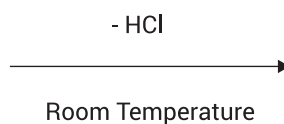


Fluorescence: Ex 340nm, Em 450 nm  
DAD: 338, 10 nm; Ref. 390, 20 nm

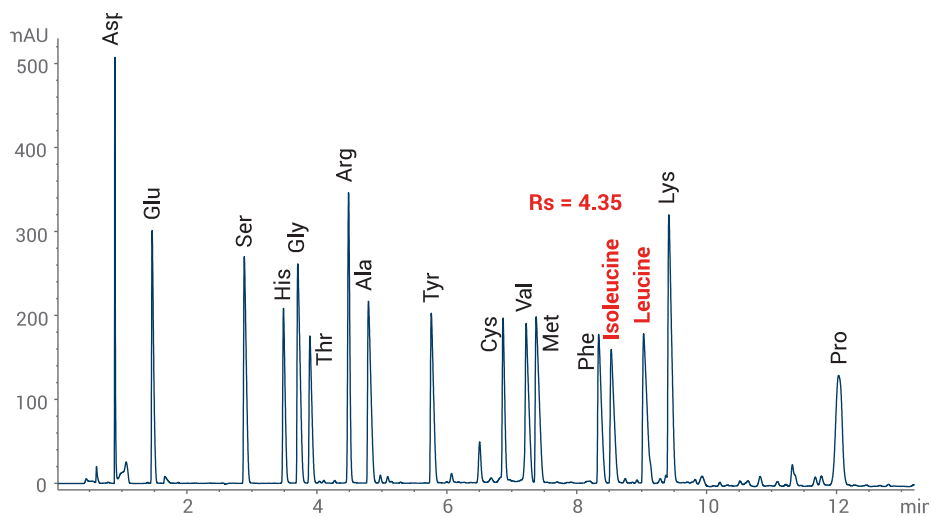
## Fluorenylmethoxy chloroformate (FMOC)



Fluorescent  
Absorbs at 262 nm and  
Fluorescences at 324 nm



Fluorescence: Ex 260 nm, Em 325 nm  
DAD: 262, 16 nm; Ref. 324, 8 nm



Ultraviolet chromatogram of amino acids from protein hydrolysate. The resolution between leucine and isoleucine is 4.35, which easily meets the European Pharmacopoeia requirement that resolution be greater than 1.5. [European Pharmacopoeia 9.0 (2.2.56) Amino Acid Analysis.]

**LC/UV**

**Column:** Agilent AdvanceBio Amino Acid Analysis, 4.6 x 100 mm, p/n 655950-802

**Column temp:** 30 °C

**Mobile phase:** Low pH, positive ion mode MS detection:  
 A = 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 10 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, pH 8.2  
 B = acetonitrile:methanol:water, 45:45:10 (v:v:v)

**Flow rate:** 1.5 mL/min

**Gradient:**

Time (min)	% B
0	2
0.35	2
13.4	57
13.5	100
15.7	100
15.7	2
18	end

**Sample:** Protein hydrolysate

**Detection:** Agilent 1260 Infinity II DAD WR



Agilent 1260 Infinity II vialsampler



Agilent 1290 Infinity II multisampler

# Analyzing underivatized amino acids and cell culture metabolites via LC/MS



Now you can analyze amino acids and other cell culture metabolites with a single method to get more information in less time.

**Performance:** PEEK-lined stainless steel columns deliver excellent peak shape and recovery of challenging ionic metabolites with pressure tolerance up to 600 bar.

**Sensitivity:** MS-friendly mobile phases provide excellent analytical sensitivity.

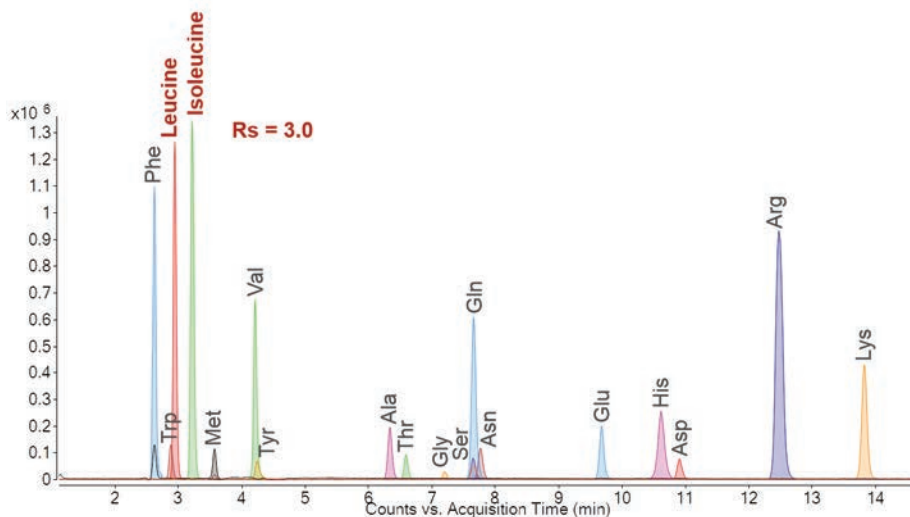
**Flexibility:** Compatible with both HPLC and UHPLC systems via 2.7  $\mu\text{m}$  Poroshell particles.

With AdvanceBio MS Spent Media columns, you don't need to derivatize your samples.

- Water soluble vitamins
- Glucose
- Lactate
- Polyamines
- And others



Use AdvanceBio MS Spent Media columns with any LC/MS system. From left: Agilent 1290 Infinity II LC system with 6545XT Q-TOF, MSD XT, and Ultivo triple quadrupole systems.



Extracted ion chromatograms of amino acids from cell culture media, collected on a time-of-flight instrument in positive ion mode. The resolution between leucine and isoleucine is 3.0, which easily meets the European Pharmacopoeia requirement that resolution be greater than 1.5. [European Pharmacopoeia 9.0 (2.2.56) Amino Acid Analysis.]

## LC/MS

**Column:** Agilent AdvanceBio MS Spent Media, 2.1 x 100 mm, part number 675775-901

**Column temp:** 30 °C

**Mobile phase:** Low pH, positive ion mode MS detection:

A = 10% 200 mM ammonium formate in water pH 3, 90% water

B = 10% 200 mM ammonium formate in water pH 3, 90% acetonitrile

Final salt concentration is 20 mM.

We recommend preparing mobile phases from a concentrated buffer stock to ensure robust and consistent mobile phases.

**Flow rate:** 0.5 mL/min

Time (min)	% B (Low pH, positive ion mode)	% B (High pH, negative ion mode)
0	100	100
15	80	80
20	100	100
20	100	100

**Sample:** Cell culture media, diluted five-fold with mobile phase B

**Detection:** Agilent 6230 time-of-flight LC/MS

## Ordering information

### AdvanceBio AAA Columns

LC/UV analysis of derivatized amino acids

Description	Part Number
AdvanceBio Amino Acid Analysis 100 Å, 3.0 x 100 mm, 2.7 µm	695975-322
AdvanceBio Amino Acid Analysis 100 Å, 4.6 x 100 mm, 2.7 µm	655950-802
AdvanceBio Amino Acid Analysis 100 Å, 3.0 x 5 mm, 2.7 µm (3-pack guards)	823750-946
AdvanceBio Amino Acid Analysis 100 Å, 4.6 x 5 mm, 2.7 µm (3-pack guards)	820750-931

### AdvanceBio AAA Standards and Reagents

Ready-to-use reagents and standards have been combined under one part number for easy ordering.

Description	Part Number
<b>Standards and Reagents kit</b>	<b>5190-9426</b>
Kit contents (can be ordered separately)	
Buffer, borate, 100 mL	5061-3339
FMOC reagent, 10 ampoules, 1 mL each, for AAA	5061-3337
OPA reagent, 10 mg/mL, 6 ampoules, 1 mL each	5061-3335
Dithiodipropionic acid (DTDPA), 5g	5062-2479
AA standard, 1 nmol 10/pk	5061-3330
AA standard, 250 pmol 10/pk	5061-3331
AA standard, 100 pmol 10/pk	5061-3332
AA standard, 25 pmol 10/pk	5061-3333
AA standard, 10 pmol 10/pk	5061-3334
AA supplement, 1 g each	5062-2478

### AdvanceBio MS Spent Media Analysis Columns

LC/MS analysis of underivatized amino acids and cell culture metabolites

Description	Part Number
AdvanceBio MS Spent Media 120 Å, 2.1 x 50 mm, 2.7 µm	679775-901
AdvanceBio MS Spent Media 120 Å, 2.1x100 mm, 2.7 µm	675775-901
AdvanceBio MS Spent Media 120 Å, 2.1x150 mm, 2.7 µm	673775-901

Learn more:

[www.agilent.com/chem/advancebio](http://www.agilent.com/chem/advancebio)

Buy online:

[www.agilent.com/chem/store](http://www.agilent.com/chem/store)

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**1-800-227-9770**

[agilent\\_inquiries@agilent.com](mailto:agilent_inquiries@agilent.com)

Europe

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Asia Pacific

[inquiry\\_lsca@agilent.com](mailto:inquiry_lsca@agilent.com)

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