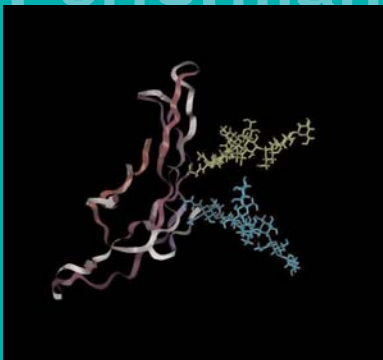




# GROWTH MEDIA

AUGUST 2004



New Products

Performance

Quality



Engineers monitor operation parameters of the tallest cryogenic distillation column in the world.

Mostly below ground, the 700 foot column is located at the Spectra Los Alamos National Laboratory facility in New Mexico, USA



Spectra Stable Isotopes (SSI) is a division of Spectra Gases, Inc, founded in 1980 to meet the needs of researchers for excimer laser gas mixtures. Spectra has since become leading supplier in next generation fine chemicals and high purity gases for the research, medical, fiber optic, semiconductor, environmental, laser, lighting, and high purity gas handling equipment markets throughout the world.

In 2001, Spectra acquired the former Stable Isotope Biochemical Division of Martek Biosciences Corporation thus gaining over 20 years of experience in employing proprietary technologies to develop and manufacture diverse isotopic materials.

Our purification systems and separation technology represent some of the most advanced processes in the world, including the world's longest continuous cryogenic distillation columns located at Spectra's ICON (**I**sotopes of **C**arbon **O**xxygen and **N**itrogen) Facility at the Los Alamos National Laboratory.

Today, Spectra Stable Isotopes is a major producer of stable isotope labeled biochemicals, synthons, and stable isotope specialty gases. Spectra is the world's largest manufacturer of deuterium, helium-3, and stable isotope labeled biochemicals.

We offer quality assured products that include growth media for bacterial, yeast, insect, and mammalian cell culture, RNA and DNA derivatives for oligonucleotide synthesis and fatty acids for metabolic studies. Also available are amino acids, protected amino acids, carbohydrates, ammonium salts, labeled solvents and reagents, and a full line of stable isotope gases.

# INDEX

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## GROWTH MEDIA

<b>PRODUCT OVERVIEW</b>	<b>3</b>
<b>GENERAL REMARKS AND INFORMATION</b>	<b>5</b>
Shelf Life and Storage Conditions	5
Aeration	5
Deuteration	5
<b>BACTERIAL CULTURE MEDIA</b>	<b>6</b>
CELSTONE® Standard Growth Media	6
CELSTONE Base Powder	8
MARTEK®9 and SPECTRA-9	9
SPECTRA 9 Medium and Base Powder	10
Basic Protocol: CELSTONE, SPECTRA-9, MARTEK 9	11
Special Formulations	12
<b>ADAPTOR KIT</b>	<b>13</b>
CELSTONE Deuteration Adaptor Kit	13
<b>OTHER MEDIA</b>	<b>14</b>
CELSTONE M and CELSTONE M-R	14
Basic Protocol: CELSTONE M	16
CELSTONE-i	17
YEASTONE 5.0	18
<b>LABELED AMINO ACID MIXTURE</b>	<b>20</b>
<b>ORDERING INFORMATION AND SUPPORT</b>	<b>22</b>
How to Order	22
Representations	23
Limited Warranty	23
Technical Support	23



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## PRODUCT OVERVIEW

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Multi-dimensional nuclear magnetic resonance (NMR) is a powerful tool in the elucidation of the three –dimensional structure of proteins in solution with applications in drug design, protein engineering and pharmacology.

Expressing  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^2\text{H}$  labeled recombinant proteins is a widely used method to obtain isotopically enriched proteins for NMR studies. The choice of expression systems includes bacterial, mammalian cell, *Saccharomyces cerevisiae* and *Pichia pastoris* yeast, baculovirus and protein synthesis in eukaryotic and proeukaryotic cell-free systems. The bacterial system is the most widely and routinely used of these expression systems.

Isotopically enriched proteins can be expressed with  $^{13}\text{C}$ ,  $^{15}\text{N}$ , or  $^2\text{H}$  individual labeling or any combination of the three. In order to facilitate the NMR researcher's needs, Spectra Stable Isotopes, offers various growth media with your choice of isotopic labeling.

CELTONE<sup>®</sup>, MARTEK<sup>®</sup>9 and SPECTRA-9 are bacterial growth media. CELTONE is a “fully rich” medium with a growth rate comparable to LB medium for most bacterial strains. MARTEK 9 and SPECTRA-9 are enhanced minimal media, which offer a cost effective alternative for labeled proteins. They all are available in any combinations of  $^{13}\text{C}$ ,  $^{15}\text{N}$ , or  $^2\text{H}$ . Refer to section titled; “Bacterial Culture Media” for more information on these three lines of media.

Spectra Stable Isotopes also provides CELTONE M and CELTONE M-R, which are mammalian cell growth media. These are serum free media and are available in different isotopic labeling as described in the section titled Other Media.

Spectra Stable Isotopes also offers CELTONE-I medium for insect cell systems, Yestone 5.0 for *Saccharomyces cerevisiae* and *Pichia pastoris* yeast systems, and Amino Acid Mixtures for cell-free synthesis.

***To support the products above, Spectra Stable Isotopes provides recommended protocols, storage and handling information.***

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**NEW**  
**Labeled Amino  
Acid Mixture**  
(see page 20)

---

Spectra Stable Isotopes now offers Labeled Amino Acid mixture for cell-free protein synthesis. The mixture contains all 20 amino acids and can be modified to meet any research requirement.

***On request, complimentary samples of many products for your prep-trial are available.***



## GENERAL REMARKS AND INFORMATION

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### **Shelf Life and Storage Conditions**

Shelf life of media is one year. Please store the media away from light at room temperature. To retain sterility, all media can be filtered through a 0.2µ Millipore® filter.

**Additionally you may autoclave CELTONE media in solution to retain sterility; but not MARTEK 9, SPECTRA-9 or deuterated media since their chemical integrity may be compromised.**

### **Aeration**

To maintain optimal aeration of your culture, use an orbital shaker apparatus with a capacity of at least 180 rpm. Fill your culture flasks to a maximum of 1/3 of the flask volume.

### **Deuteration**

Most *E. coli* strains do not need careful adaptation to a deuterated environment when using growth media containing amino acids, since the "stress" for the cells is reduced compared to glucose minimal media. Growth is typically slower, compared to non-deuterated media and induction time needs to be adapted.

However, if you cannot achieve satisfactory cell growth, or expression of the protein in deuterated medium, we can provide CELTONE, MARTEK and SPECTRA 9 Deuteration Adaptor Kits which will help you achieve superior expression.

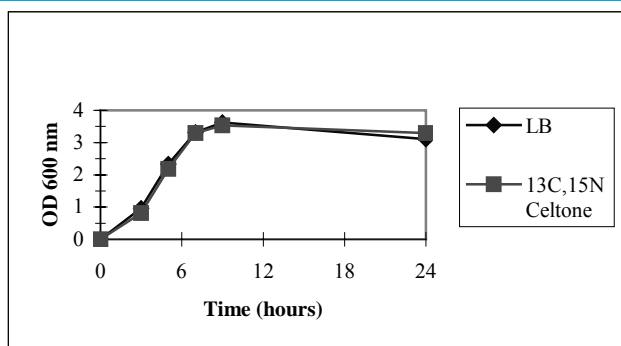
# BACTERIAL CULTURE MEDIA

## CELTONE<sup>®</sup> Standard Growth Medium

### Product Description

CELTONE is a “rich” bacterial cell growth medium derived from an algal source with a growth rate comparable to LB allowing for inoculation and induction within one working day. CELTONE contains amino acids, nucleic acids, peptides, vitamins, salts and other essential nutrients and provides excellent cell growth and high protein expression.

It is recommended that researchers interested in growing bacterial cultures with our CELTONE medium perform a small-scale prep trial before conducting large-scale experiments.



<sup>13</sup>C<sup>15</sup>N labeled CELTONE medium vs. LB medium (on E. coli BL21)

CELTONE is available in solution and powder forms and can be supplied with <sup>13</sup>C, <sup>15</sup>N, or <sup>2</sup>H individual labeling or any combination of the three. For each lot of CELTONE, isotopic purity and enrichment are tested by advanced analytical techniques and is typically ≥ 98%. Partially <sup>2</sup>H labeled media are also available upon request.

CELTONE is formulated as ready-to-use medium without need for dilution. Each lot of CELTONE medium is tested for sterility, cell growth and protein expression to ensure the quality of the product. An analytical certificate is provided with each shipment.

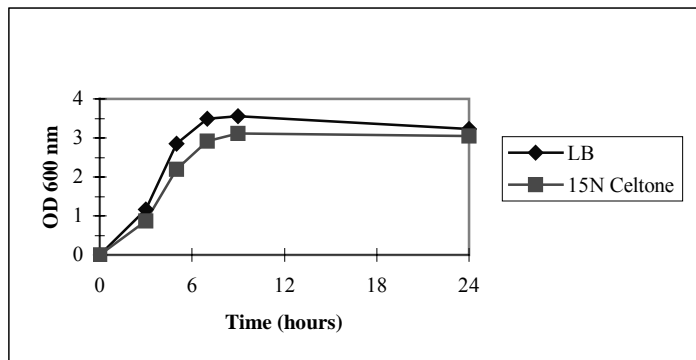
Following is a typical CELTONE amino acid profile table.

FREE AMINO ACID COMPOSITION				TOTAL AMINO ACID COMPOSITION			
Ala:	11.1%	Arg:	4.6%	Ala:	7.6%	Arg:	5.6%
Asp:	17.5%	Glu:	10.0%	Asp:	9.6%	Glu:	10.2%
Gly:	11.5%	His:	1.2%	Gly:	6.4%	His:	2.3%
Ile:	1.3%	Leu:	7.7%	Ile:	3.1%	Leu:	8.4%
Lys:	7.1%	Met:	1.7%	Lys:	12.0%	Met:	1.6%
Phe:	3.8%	Pro:	6.2%	Phe:	8.3%	Pro:	5.6%
Ser:	5.2%	Thr:	2.8%	Ser:	4.4%	Thr:	4.8%
Tyr:	3.7%	Val:	2.1%	Tyr:	3.8%	Val:	4.5%
Trp:	0.2%	Cys:	0.2%	Trp:	0.2%	Cys:	0.7%

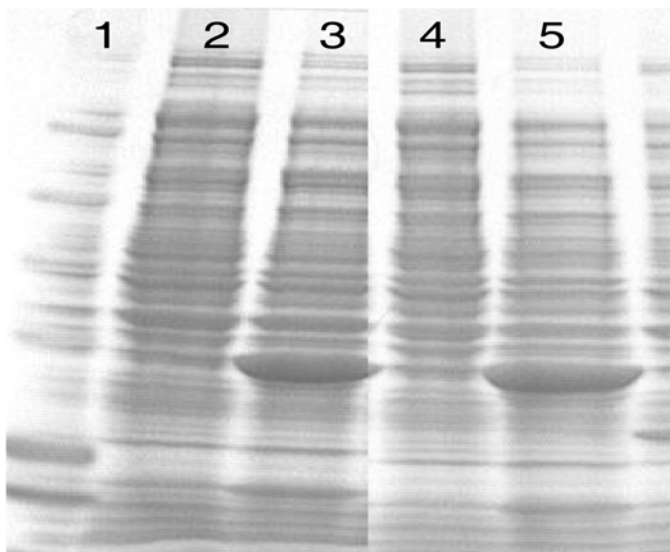
Analytical data was obtained on a specific batch of <sup>13</sup>C<sup>15</sup>N labeled CELTONE (CB0041).

*Contact us for a complimentary sample of unlabeled CELTONE medium.*

## CELTONE Media



<sup>15</sup>N labeled CELTONE medium vs. LB medium (on E.coli BL21)



Comparison of the expression of a 20 kDa protein in CELTONE versus LB medium

Lane 1: Molecular weight ladder  
Lane 2: uninduced protein from LB medium  
Lane 3: IPTG induced protein from LB medium  
Lane 4: uninduced protein from CELTONE medium  
Lane 5: IPTG induced protein from CELTONE medium

### References:

1. <sup>15</sup>N -labelling and preliminary heteronuclear NMR study of *Desulfovibrio vulgaris* Hildenborough cytochrome c553. Morelli X, Dolla A, Toci R, Guerlesquin F. *Eur J Biochem* 1999 Apr, 261(2): 398-404
2. Expression of doubly labeled *Saccharomyces cerevisiae* iso-1 fer-ricytochrome c and 1H, 13C and 15N chemical shift assignments by multidimensional NMR. Christina M. Szabo, Lori K, Sanders, H, Carl Le, Ellen Y.T, Chien, Eric Oldfield. *FEBS letters* 482 (2000) 25-30
3. Binding ability of HHP. Tagged proteins towards Ni<sup>2+</sup> studied by paramagnetic NMR relaxation. Jensen, Lauritzen, Dahl, Rederson, et. al. *Journal of Biomol NMR* 29: 175-185, 2004

## CELTONE<sup>®</sup> Base Powder

CELTONE is also available in powder form - CELTONE Base Powder. The advantage of this medium is its versatility as researchers can formulate their own medium using CELTONE Base Powder, based on their protein protocol. Typically, a minimum of 10 grams of CELTONE Base Powder is used to reach comparable LB densities.

CELTONE Base Powder contains amino acids, peptides, vitamins and other essential nutrients, which allow the researchers to customize the medium for their research protocols.

### CELTONE Powder Analytical Information

#### APPROXIMATE COMPOSITION

Amino Acids	70%
Sodium Chloride	20%
Moisture	10%
Glucose	<0.5%
Other Sugars	Trace

Each lot of CELTONE Powder is tested for cell growth and protein expression.

### Reconstitute Your Own Medium with CELTONE Base Powder

CELTONE Base Powder is available in <sup>13</sup>C, <sup>15</sup>N, or <sup>2</sup>H individual labeling or in any combination of the three. Each lot of CELTONE Base Powder is tested for isotopic purity and enrichment by advanced analytical techniques and is typically ≥ 98%.

Partially <sup>2</sup>H labeled medium is also available upon request.

*Please contact us if you need more information about CELTONE Base Powder*

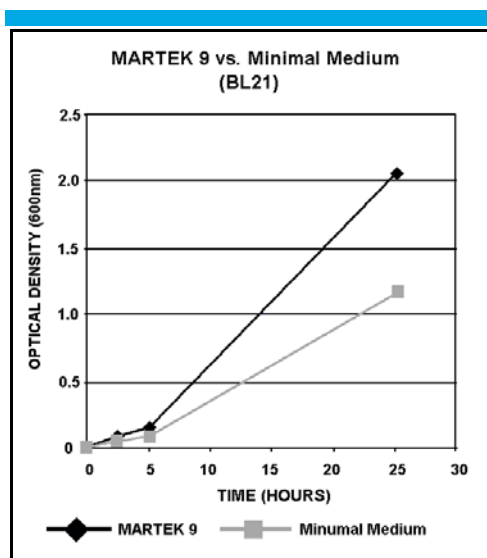
# MARTEK<sup>®</sup> 9 and SPECTRA-9

## Product Description and Performance

MARTEK 9 and SPECTRA-9 are cost effective media for E.coli bacterial growth and protein expression. They contain <sup>15</sup>N labeled salts and <sup>13</sup>C labeled carbohydrates, which allow for the expression of <sup>13</sup>C, <sup>15</sup>N labeled proteins in bacterial expression systems. Both media are also supplemented with CELTONE Base Powder, which contains amino acids, vitamins, peptides and other essential nutrients to ensure optimal cell growth and protein expression for most expression systems.

### MARTEK 9

MARTEK 9 is formulated as ready-to-use medium and is an excellent source for bacterial cell growth and protein expression.



MARTEK 9 Medium vs.  
M9 Minimal Medium (with 4g/L glucose)

A comparison of the growth between MARTEK 9 and M9 minimal medium (left) shows that cultures grown in MARTEK 9 grew much better.

Spectra Stable Isotopes can supply MARTEK 9 with <sup>13</sup>C, <sup>15</sup>N, or <sup>2</sup>H individual labeling or in any combination of the three. For each lot of MARTEK 9, isotopic purity and enrichment are tested by advanced analytical techniques and is typically  $\geq 98\%$ . MARTEK 9 medium is also tested for sterility, growth and protein expression levels to ensure the quality of the product. An analytical certificate is provided with each shipment.

### References:

1. Letters to the Editor, Backbone 1, 15, 13resonance assignments of  $\alpha$ -ADT &  $\beta$ ADT. John, Heller, Coles, Bosch, Banmaster, Kessler. *Journal of Bio-Mol NMR* Vol. 29 number 2 – June 2004

*Partially <sup>2</sup>H labeled medium is also available upon request.*

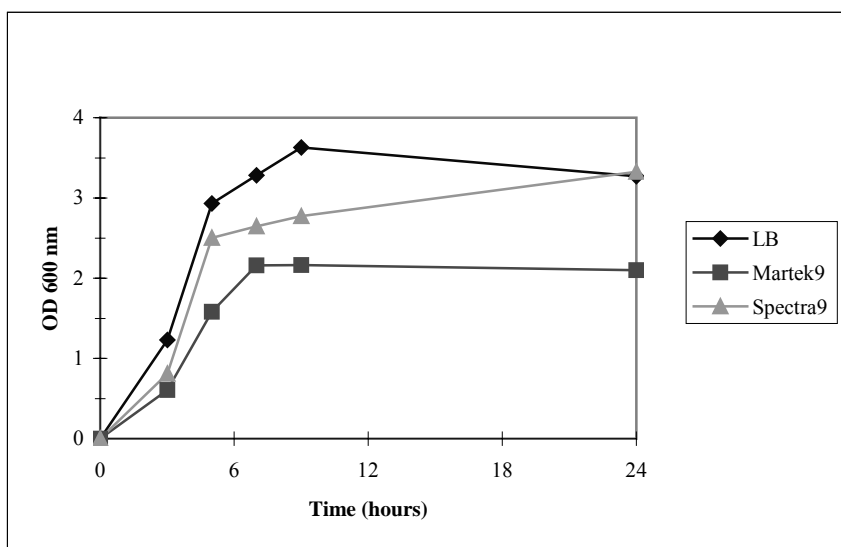
## SPECTRA-9

### New Product

In order to meet the growing needs of the NMR research community, Spectra Stable Isotopes has recently introduced a new growth medium, SPECTRA-9. This media is an improved version of MARTEK 9, supplemented with additional nutrients, resulting in superior growth and higher protein expression versus MARTEK 9.

SPECTRA-9 medium is available as ready-to-use and can be supplied with  $^{13}\text{C}$ ,  $^{15}\text{N}$ , or  $^2\text{H}$  individual labeling or any combination of the three.

For each lot of SPECTRA-9, isotopic purity and enrichment are tested by advanced analytical techniques and is typically  $\geq 98\%$ . SPECTRA-9 medium are also tested for sterility, growth and protein expression levels to ensure the quality of the product. An analytical certificate is provided with each shipment.



$^{13}\text{C}$ ,  $^{15}\text{N}$  Spectra 9 vs.  $^{13}\text{C}$ ,  $^{15}\text{N}$  MARTEK 9 vs. LB medium (on E.coli BL21)

*A complimentary sample of unlabeled SPECTRA-9 available upon request.*

## Basic Protocol

### CELTONE SPECTRA 9 MARTEK 9

This is the basic protocol for protein expression using CELTONE, MARTEK 9 or SPECTRA-9 growth media. Before preparing large scale labeled cultures, we recommend testing the growth and expression rates on a small-scale (3ml) sample. This helps ensure optimal expression of recombinant protein.

#### To prepare 1 liter of medium:

##### Day 1

1. Prepare a 20 ml starter culture, preferably overnight.
  - a. Allow medium to warm to room temperature (~21° C).
  - b. Add proper concentrations of desired antibiotics.
  - c. Inoculate with cells from a single colony on an agarose plate or from a cell cryo-stock.
  - d. Allow cells to incubate at 37°C, shaking at ~250 rpm for a minimum period of 12 hours, or overnight.

##### Day 2

2. Prepare a one-liter production culture.
  - a. Add proper concentrations of desired antibiotics to one liter of media.
  - b. Inoculate by adding the entire contents of the 20 ml starter culture into the one liter of media above.
  - c. Allow cells to incubate at 37°C, shaking at ~250 rpm.
3. Monitor the cell culture growth.

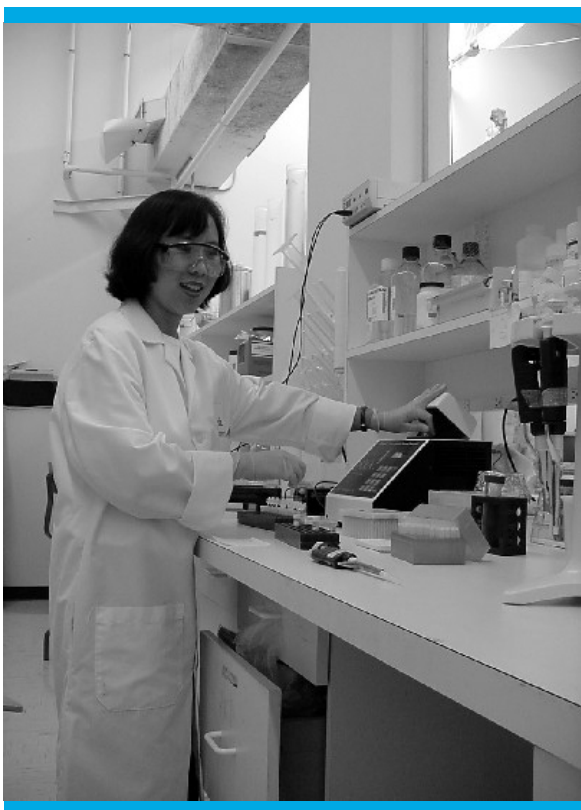
Use a photospectrometer set at 600 nm to measure the optical density (OD 600) of the culture during growth. Once the optical density of the culture has reached (OD 600, 0.8-1.0), the culture is ready for induction.
4. Induction of the culture
  - a. Induce the protein expression by adding the appropriate amount of desired induction chemical.
  - b. Allow cells to incubate at 37°C for another 2-4 hours.
5. Harvest the completed cell culture.
  - a. Pour the culture into a one-liter centrifuge bottle.
  - b. Centrifuge for 20 minutes.
  - c. Gently pour off the cleared medium supernatant.
  - d. The pelleted cells are now ready for further processing.

*NOTE: Depending on the condition of your target protein, it may be possible to freeze the cell pellet until further processing.*

## Special Formulations

Spectra continues to develop, improve and provide quality bacterial growth media to fulfill the ever-changing needs of researchers throughout the world. We can provide bacterial growth medium selectively labeled with a single amino acid or multiple labeled amino acids for protein expression to suite your specific research protocols.

We invite you to contact our technical team for any questions on issues related to growth conditions, induction, or protein expression.



### ***References:***

Methyl side-chain Dynamics in Proteins Using Selective Enrichment with a Single Isotopomer, Michael M. Chaykovski, Lynnette C. Bae, Minn-Chang Cheng, Jenny H. Murray, Kenneth E. Tortolani, Rui Zhang, Kothandaraman Seshadri, John H. B. C. Findlay, Shih-Yang Hsieh, Arnout P. Kalverda, Steve W. Homans, and Jonathan miles Brown. *J. AM. CHEM. SOC.*2003, 125,15767-15771

## ADAPTOR KIT

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### CELTONE Deuteration Adaptor Kit

We recommend a deuteration adaptor kit for best results with our deuterium labeled CELTONE products. The adaptor kit can be modified to suit your needs, so we invite you to contact us for further information.

The kit contains 100 mls of each of the following media:

- Unlabelled CELTONE
- 25% d-CELTONE
- 50% d-CELTONE
- 75% d-CELTONE
- Fully deuterated CELTONE

We recommend the following protocol as a start. You can use this kit as a basis, and follow your preferred protocol.

1. Inoculate a sample of the unlabelled media using your protocol. This may be via a chip of frozen glycerol stock, or a single colony from a plate.
2. Incubate the culture at 37 °C overnight or until the OD reaches >0.5. Use 1 ml of this culture to inoculate a 25 ml volume of 25% or 50% deuterated media.
3. Grow the culture at 37 degrees until the OD > 0.5.
4. Use 1 ml of above culture to inoculate a 25 ml volume of 75% deuterated media and grow the culture at 37 °C until the OD > 0.5. Please note that these cultures may take increasingly longer amounts of time to grow due to gradual deuterium enrichment.
5. Using 2.5 ml of the 75% culture, inoculate a 25 ml volume of 97% media and incubate at 37° C until OD is > 0.5.
6. After confirming all quality criteria, use this sample to inoculate a larger volume of fully deuterated media, for example, 10 ml into a one liter, fully deuterated medium, for your protein expression experiment.

Spectra provides 100 mls of labeled CELTONE, MARTEK 9 and SPECTRA-9 media so that you may have the opportunity to re-inoculate a small sample at any of the above steps to produce a culture of higher enrichment.

## OTHER MEDIA

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### **CELTONE M** Product and Description

#### *An Isotopically Labeled Mammalian Cell Growth Medium*

The Mammalian cell expression system is an important method for NMR studies of proteins because it provides a unique protocol for the production of proteins in a fully post-translationally modified state.

Spectra Stable Isotopes offers two versions of mammalian cell growth media, CELTONE M and CELTONE M-R.

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#### *Two Versions of Mammalian Cell Growth Media are Available: CELTONE M and CELTONE M-R*

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#### **CELTONE M**

CELTONE M is a serum free medium, modified from CHO-S-SFM II (Invitrogen). It is a chemically defined medium, augmented with our labeled amino acid mixture, other nutrients and  $^{13}\text{C}$  labeled carbohydrates to optimize the growth of mammalian cells and expression of recombinant proteins.

#### **CELTONE M-R**

CELTONE M-R is also a serum free chemically defined medium, augmented with labeled amino acids and  $^{13}\text{C}$  labeled carbohydrates. It is similar to traditional RPMI (Roswell Park Memorial Institute) medium. The cell growth and protein expression is comparable when using the same cell lines.

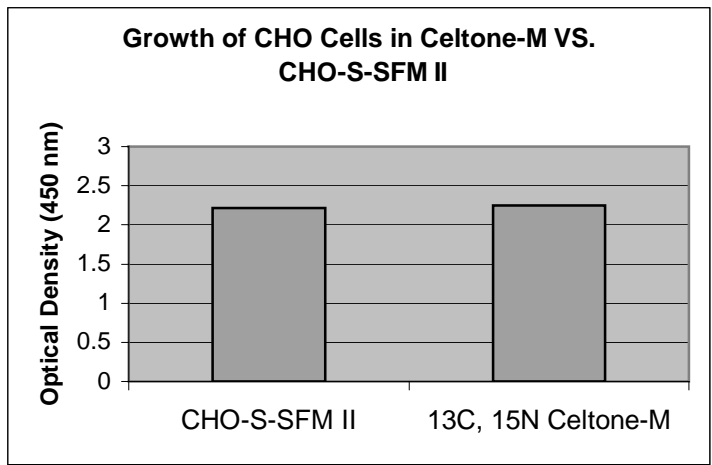
#### **Quality Control:**

#### **CELTONE M & CELTONE M-R**

All amino acids mixtures and  $^{13}\text{C}$  labeled carbohydrates in CELTONE M and CELTONE M-R are tested for the isotopic enrichment and chemical purity through advanced analytical techniques and is typically  $\geq 98\%$ . Media growth is also compared to CHO-S-II SFM medium (Invitrogen) as seen in Figure 6.

CELTONE M and CELTONE M-R can be supplied in  $^{13}\text{C}$ , or  $^{15}\text{N}$  individual labeling or  $^{13}\text{C}$ , and  $^{15}\text{N}$  labeling.

*Contact us for a complimentary sample of unlabeled CELTONE M and/or CELTONE M-R.*



**FIGURE 6**  
Growth of the media is compared to CHO-S-II SFM medium (Invitrogen) for Quality Assurance

Customized mammalian cell culture medium is also available from Spectra. Please contact us for more information.



# Basic Protocol

## CELTONE M

**This is the reference protocol for expressing proteins in CELTONE M growth media as suspension culture.**

Before preparing large scale labeled cultures, we recommend testing the growth and expression on a small scale. This will ensure optimal expression of the recombinant protein.

1. Proceed with an unlabelled medium first and adapt the mammalian cells to serum free medium.
  - **Direct Adaptation.** Many cell lines such as CHO cells can go directly from growth in serum-supplemented medium into CELTONE M medium without any problems.
  - **Sequential Adaptation.** Cells switch from serum-supplemented medium into serum free medium in several steps (please refer to the standard adaptation protocol if you need to do so).
2. Pellet adapted mammalian cells by centrifuge down at low speed (120-150 rpm) for 10 –15 minutes.
3. Re-suspend cells in PBS using 10 % volume of original culture.
4. Centrifuge cells at low speed.
5. Re-suspend cell pellet in a volume of CELTONE M media so that the viable cell concentration is approximately  $1 \times 10^6$  cells/ml.
6. Incubate culture at 37<sup>0</sup>C in a humidified atmosphere of 5 %-8% CO<sub>2</sub> for 24 hours.
7. Centrifuge the culture. Wash resulting cell pellet with PBS.
8. Re-suspend the cells in the same volume of CELTONE M medium as used in previous culture. (Step 7 and 8 are optional)
9. Incubate culture at 37<sup>0</sup>C in a humidified atmosphere of 5%-8% CO<sub>2</sub>.
10. Monitor expression levels depending on expression of target protein.

**CELTONE-i**  
*An Isotopically  
Labeled  
Insect Cell  
Growth Medium*

**Product and Description**

Baculoviruses are powerful expression systems used for the production of biologically active recombinant proteins. Insect cells have been the choice for both expression of recombinant proteins using baculovirus expression systems.

CELTONE-i is a serum-free, yeastolate-free medium, supplemented with our labeled amino acid mixture, other nutrients and  $^{13}\text{C}$  labeled carbohydrates. This allows production of labeled recombination proteins for SF9, High 5 and other insect cell lines.

**Quality Control: CELTONE-i**

All amino acid mixtures and  $^{13}\text{C}$  labeled carbohydrates comprising CELTONE-i medium, are tested for isotopic enrichment by LCMS, GC/MS and is typically  $\geq 98\%$ . The cell density of SF9 cells in each lot of CELTONE-i is measured over a seven-day period to ensure quality and stability of this product.

**INTRODUCING**  
*CELTONE<sup>®</sup>i, the Premier Isotopically  
Labeled Insect Cell Medium  
Specifically Formulated for  
Production of Labeled Proteins.*

CELTONE-i is available in  $^{13}\text{C}$ , or  $^{15}\text{N}$  individual labeling or both  $^{13}\text{C}$ , and  $^{15}\text{N}$  labeling.



*Contact us for a complimentary sample of unlabeled CELTONE-i.*

**YEASTONE 5.0**  
*An Isotopically  
Labeled  
Growth Medium for  
S. cerevisiae and  
P. pastoris*

**Product and Description**

*Saccharomyces cerevisiae* and *Pichia pastoris* are widely used eukaryotic model organisms due to extensive understanding of their molecular biology. The simplicity with which *Saccharomyces cerevisiae* and *Pichia pastoris* can be manipulated and cultured renders them as two of the premier eukaryotic organisms for generating recombinant proteins.

Yeastone 5.0 is a synthetic medium, which allows for rapid growth of *Saccharomyces cerevisiae* or *Pichia pastoris*. It contains a proprietary mixture of defined growth promoting ingredients and is free of any amino acids and proteins.

Yeastone 5.0-P (*Pichia pastoris* medium) is a methanol inducible medium that can be formulated to contain  $^{13}\text{C}$ , or  $^{15}\text{N}$  individual labeling, or both  $^{13}\text{C}$ , and  $^{15}\text{N}$  labeling. If needed, we provide additional methanol to our research clients to add to cultures as a continuation base to maintain induction.

Yeastone 5.0-S (*Saccharomyces cerevisiae* medium) contains either galactose or glucose depending upon your requirements. Since it is an amino acid free medium, it is compatible with both antibiotic as well as auxotrophy-based selection systems. For glucose medium, Yeastone 5.0-S can be formulated with  $^{13}\text{C}$ , or  $^{15}\text{N}$  individual labeling, or both  $^{13}\text{C}$ , and  $^{15}\text{N}$ , labeling. At this time, the galactose medium can only be formulated with  $^{15}\text{N}$  labeling.

*Contact us for a complimentary sample of unlabeled medium.*



# LABELED AMINO ACID MIXTURES

## for cell-free protein synthesis

---

### Amino Acid Mixture

New Product

### Product and Description

Cell-free protein synthesizing systems have been used extensively for the in-vitro translation of proteins. In combination with the development of the *in-vitro* transcription system, cell-free translation systems allow us to demonstrate a direct correlation between a DNA sequence and the encoded polypeptides.

Spectra Stable Isotopes now offers a new product, Amino Acid Mixture, for cell-free protein synthesis. The mixture contains all 20 amino acids and can be modified to meet various research needs. It can be used on commercially available eukaryotic and prokaryotic translation systems.

Each lot of labeled Amino Acid Mixture is tested for  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^2\text{H}$  isotopic enrichment by LCMS and NMR, and is typically  $\geq 98\%$ . Each lot of Amino Acid Mixture is also tested by performing coupled in vitro transcription/translation of circular DNA template reaction using E. coli T7 S30 Extract System for Circular DNA (Promega) to ensure the quality and stability of this product. See figure 8.

### INTRODUCING Amino Acid Mixture for Cell-Free Protein Synthesis

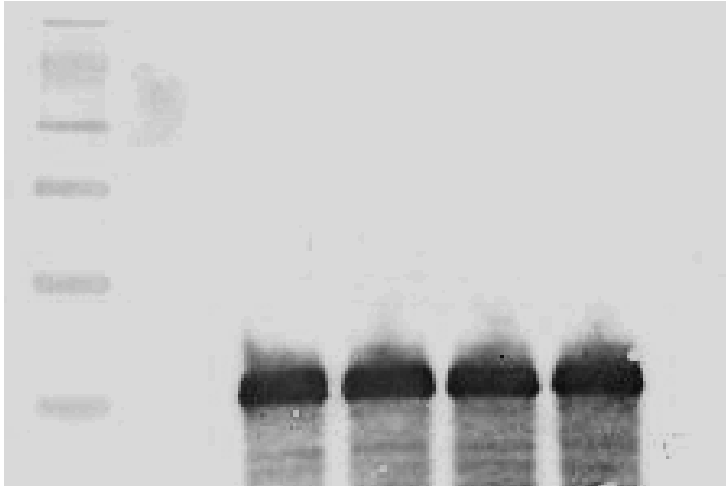
Spectra can supply the Amino Acid Mixture in  $^{13}\text{C}$ ,  $^{15}\text{N}$ , or  $^2\text{H}$  individual labeling or a combination of the three.

### Amino Acid Mixture Profile

Amino Acid	~Content %	Amino Acid	~Content %
Alanine	6.9	Leucine	7.6
Arginine	5.1	Lysine	10.9
Asparagine	3.6	Methionine	1.4
Aspartic Acid	8.7	Phenylalanine	7.5
Cysteine	3.6	Proline	5.1
Glutamine	3.6	Serine	4.0
Glutamic Acid	9.2	Threonine	4.3
Glycine	5.8	Tryptophan	0.2
Histidine	2.1	Tyrosine	3.4
Isoleucine	2.8	Valine	4.1

**FIGURE 8**

Coupled in vitro transcription/translation of circular DNA template reaction using E. Coli T7 S30 Extract System for Circular DNA (Promega) with labeled Amino Acid Mixture



Lane 1: molecular weight ladder  
Lane 2: negative control without DNA template  
Lane 3: reaction with amino acid mix from Promega.  
Lane 4: reaction with unlabeled Spectra amino acid mixture  
Lane 5: reaction with  $^{15}\text{N}$  labeled Spectra amino acid mixture  
Lane 6: reaction with  $^2\text{H}$ ,  $^{15}\text{N}$  labeled Spectra amino acid mixture

## ORDERING INFORMATION AND SUPPORT

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### How To Order

Orders may be placed by phone, fax, or online.

For domestic orders: 1.800.338.9959.

For international orders: 1.301.776.9029 (ext.100)  
between 8:00 a.m. and 5:00 p.m. Eastern Time.

For fax orders: 1.410.740.1328

You may also order directly from our website at:

***www.SpectraStableIsotopes.com.***

Phone orders need to be confirmed in writing. To help expedite shipment of your order, please make sure to include:

- Shipping Address
- Billing Address
- Purchase Order Number
- User Name and Phone Number
- Product Name
- Quantity
- Quoted Price
- Unit Package Size

### Prices

For quantity discounts, annual contracts, or non-catalog quantities, please contact Spectra Stable Isotopes Customer Service Department. Firm written quotations can be sent if requested.

### Payment Terms

All payments must be remitted in U.S. Dollars, net 15 days from invoice date, with prior credit approval. Past due invoices will be subject to a 1.5% per month (18% per year) service charge. Spectra reserves the right to request advance payment or COD payment on certain initial orders.

### Quotations

All quotations are valid for 60 days from the date of assurance.

### Shipping & Handling

Shipping charges are invoiced F.O.B. Columbia, MD, USA. Routine shipments are sent via Federal Express or UPS. Other shippers can be used upon customer request. For international shipments, all import duties and taxes are the responsibility of the customer.

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### Return Policy

Products are guaranteed to meet specifications stated at the time of ordering for a period of 30 days following the date of shipment. It is the responsibility of the user to approve the quality and quantity of the product within that time period. Spectra's liability shall be limited to issuing credit in the amount of the purchase price or replacement, at no charge, of the product. Spectra must approve all returns therefore we cannot issue credit for products returned without prior approval. Returns not due to error on Spectra's part shall be subject to a 15% restocking fee. In the case of replacement or refund, Spectra will require that the unacceptable product be returned. Please contact Spectra Stable Isotopes to obtain a return merchandise authorization number.

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## Representations

### CLINICAL OR MEDICAL USE OF PRODUCTS:

The products offered by Spectra in this catalog are for research purposes only. Spectra supplies some products in sterile and pyrogen-free state as part of special research requirements. This does not imply suitability for human use. Spectra may be able to facilitate in obtaining a permit to use its products for human applications by supplying quality control and manufacturing protocols to regulatory bodies or agencies upon request.

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## Limited Warranty

Except as stated and provided in the invoice, Spectra expressly disclaims any warranties of merchantability or fitness for a particular purpose, or any other expressed or implied warranty. Spectra's maximum liability for any reason shall be replacement of the product or refund of the purchase price. In no event shall Spectra be liable for special, indirect, or consequential damages, whether or not caused or resulting from Spectra negligence.

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## Technical Support

At Spectra Stable Isotopes quality is our first priority. All of our products go through vigorous quality control to ensure reliability. For any questions concerning our products, please contact us at [www.spectrastableisotopes.com](http://www.spectrastableisotopes.com) or call us at 410.740.7381.

## NOTES

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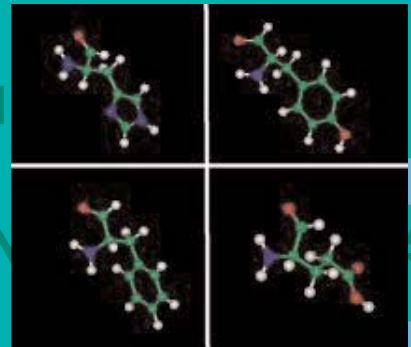




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