

3301 Kinsman Boulevard  
Madison, WI 53704

**Certificate of Analysis**

Final Report

**National Institutes of Health**

BLDG 14A, RM 119A8  
Bethesda Maryland 20892 United States

|                            |                             |                               |                                      |
|----------------------------|-----------------------------|-------------------------------|--------------------------------------|
| <b>Client Sample Name:</b> | <b>Zebra Fish Diet 0409</b> | <b>Covance Sample Number:</b> | <b>186729</b>                        |
| <b>Project ID</b>          | NAT_INST-20090504-0006      | <b>Receipt Date</b>           | 04-May-2009                          |
| <b>PO Number</b>           | Charge - AMEX               | <b>Login Date</b>             | 04-May-2009                          |
|                            |                             | <b>Storage Condition</b>      | -20 (+/- 10) Degrees Celsius         |
|                            |                             | <b>Number Composited</b>      | 1                                    |
|                            |                             | <b>Disposal Instructions</b>  | Dispose 60 days after final reported |

| <b>Analysis/Result</b>                 | <b>Result</b> |
|----------------------------------------|---------------|
| <b>Choline</b>                         |               |
| Choline                                | 3130 ppm      |
| <b>Fat</b>                             |               |
| Fat                                    | 17.3 %        |
| <b>Crude Fiber</b>                     |               |
| Crude Fiber                            | 0.7 %         |
| <b>Protein (N x 6.25) Dumas Method</b> |               |
| Protein                                | 53.7 %        |
| <b>Vitamin A</b>                       |               |
| Vitamin A                              | 25700 IU/kg   |
| <b>Vitamin D</b>                       |               |
| Vitamin D                              | 7620 IU/kg    |
| Vitamin D2                             | <200 IU/kg    |
| <b>Vitamin E</b>                       |               |
| Vitamin E                              | 477 IU/kg     |
| <b>Thiamin</b>                         |               |
| Thiamin                                | 43.2 ppm      |
| <b>Riboflavin</b>                      |               |
| Riboflavin                             | 141 ppm       |
| <b>Niacin</b>                          |               |
| Niacin                                 | 694 ppm       |
| <b>Pyridoxine Hydrochloride</b>        |               |
| Pyridoxine Hydrochloride               | 74.9 ppm      |
| <b>Folic Acid</b>                      |               |
| Folic Acid                             | 11.9 ppm      |
| <b>Vitamin B12</b>                     |               |
| Vitamin B12                            | 168 mcg/kg    |
| <b>Biotin</b>                          |               |
| Biotin                                 | 1.26 ppm      |
| <b>Pantothenic Acid</b>                |               |
| Pantothenic Acid                       | 212 ppm       |
| <b>Iodine</b>                          |               |
| Iodine                                 | 2.89 ppm      |
| <b>Selenium</b>                        |               |

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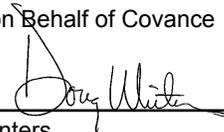
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| <b>Client Sample Name:</b> | <b>Zebra Fish Diet 0409</b> | <b>Covance Sample Number:</b> | <b>186729</b>                        |
| <b>Project ID</b>          | NAT_INST-20090504-0006      | <b>Receipt Date</b>           | 04-May-2009                          |
| <b>PO Number</b>           | Charge - AMEX               | <b>Login Date</b>             | 04-May-2009                          |
|                            |                             | <b>Storage Condition</b>      | -20 (+/- 10) Degrees Celsius         |
|                            |                             | <b>Number Composited</b>      | 1                                    |
|                            |                             | <b>Disposal Instructions</b>  | Dispose 60 days after final reported |

| <b>Analysis/Result</b>                       | <b>Result</b>  |
|----------------------------------------------|----------------|
| <b>Selenium</b>                              |                |
| Selenium                                     | 2.48 ppm       |
| <b>Elements by ICP Emission Spectrometry</b> |                |
| Calcium                                      | 2.39 %         |
| Copper                                       | 66.6 ppm       |
| Iron                                         | 252 ppm        |
| Magnesium                                    | 0.167 %        |
| Manganese                                    | 75.5 ppm       |
| Phosphorus                                   | 1.59 %         |
| Potassium                                    | 0.606 %        |
| Sodium                                       | 0.517 %        |
| Zinc                                         | 119 ppm        |
| <b>Ash</b>                                   |                |
| Ash                                          | 9.52 %         |
| <b>Moisture</b>                              |                |
| Moisture                                     | 4.4 %          |
| <b>L-ascorbyl-2-phosphate</b>                |                |
| L-ascorbyl-2-phosphate                       | 805 ppm        |
| <b>Clostridium perfringens</b>               |                |
| Clostridium Perfringens                      | <10 CFU/g      |
| <b>Escherichia coli Count</b>                |                |
| Escherichia Coli                             | <10 CFU/g      |
| <b>Salmonella BAM (Rapid method)</b>         |                |
| Salmonella                                   | Negative /25 g |
| <b>Yeast and Mold Count</b>                  |                |
| Yeast Count                                  | <10 CFU/g      |
| Mold Count                                   | <10 CFU/g      |

Signed on Behalf of Covance



Doug Winters  
Laboratory Director

**For questions on this report, please contact your Client Service Representative**

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BLDG 14A, RM 119A8  
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### Method References

### Testing Location

#### Ash (ASHM\_S:3)

Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 923.03.

Covance Laboratories Inc.

#### Biotin (BIOM\_S:11)

Scheiner, J. and De Ritter, "Biotin Content of Feedstuffs," Journal of Agricultural Food Chemistry, 23(6):1157-1162 (1975). (Modified)

Wright and Skeggs, Procedures of the Society of Experimental Biology and Medicine, 56:95, (1944). (Modified)

Methods of Analysis for Infant Formulas, Infant Formula Council, (1985). (Modified)

Journal of the AOAC, 49:882, (1996). (Modified)

Covance Laboratories Inc.

#### Choline (COL4\_S:7)

Covance Laboratories Inc.

#### Clostridium perfringens (CLOS:2)

1. Compendium of Methods for the Microbiological Examination of Foods. Clostridium perfringens, 4th Edition, Chapter 34, 2001. American Public Health Association: Washington D.C. Modified.
2. Bacteriological Analytical Manual, Clostridium perfringens, Chapter 16, 2001 Food and Drug Administration, AOAC International: Gaithersburg, Maryland. Modified.
3. Clostridium perfringens in Foods: AOAC Official Method 976.30, Official Method of Analysis of the AOAC International, 18th Edition, 2005. Gaithersburg, Maryland. Modified.

Covance Laboratories Inc.

#### Crude Fiber (CFIB\_S:2)

Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 962.09.

Covance Laboratories Inc.

#### Elements by ICP Emission Spectrometry (ICP\_S:11)

Official Methods of Analysis of AOAC INTERNATIONAL, (2005) 18th ED., AOAC INTERNATIONAL Gaithersburg, MD, USA, Official Methods 984.27, 985.01.

Covance Laboratories Inc.

#### Escherichia coli Count (COLC:4)

Compendium of Methods for the Microbiological Examination of Foods, Colony Count Methods, 4th Edition, Chapter 6,7, American Public Health Association: Washington, D.C. (2001). Modified.

Covance Laboratories Inc.

#### Fat (FAAH\_S:5)

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 922.06 and 954.02, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

Covance Laboratories Inc.

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### Method References

### Testing Location

#### Folic Acid (FOAN\_S:11)

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 960.46 and 992.05, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

Methods of Analysis for Infant Formulas, Infant Formula Council, Atlanta, GA, Section C-2, (1985).

Covance Laboratories Inc.

#### Iodine (IOL\_S:3)

Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th ED., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 932.21. (Modified)

Binnerts, W.T., Analytical Chemistry, 10:78, (1954). (Modified)

Heerspink, W., Op Deweegh, G.J., Clinical Chimica Acta, 39:327-338, (1971). (Modified)

Covance Laboratories Inc.

#### L-ascorbyl-2-phosphate (A2P\_S:1)

Covance Laboratories Inc.

#### Moisture (M100T100\_S:3)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 925.09 and 926.08, AOAC INTERNATIONAL, Gaithersburg, MD, USA,(2005).

#### Niacin (NIAP\_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 944.13 and 960.46, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005)

#### Pantothenic Acid (PANN\_S:10)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 945.74 and 960.46, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005)

#### Protein (N x 6.25) Dumas Method (DGEN\_S:3)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 968.06 and 992.15, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

#### Pyridoxine Hydrochloride (B6A\_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 961.15, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

Atkins, L., Schultz, A. S., Williams, W. L., and Frey, C. N., "Yeast Microbiological Methods for Determination of Vitamins," Industrial and Engineering Chemistry, Analytical Edition, 15:141-144, (1943).

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BLDG 14A, RM 119A8  
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### Method References

### Testing Location

#### Riboflavin (B2FV\_S:9)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 940.33 and 960.46, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

The United States Pharmacopeia, 29th Ed., p. 1913, United States Pharmacopeial Convention, Inc.: Rockville, Maryland (2005).

#### Salmonella BAM (Rapid method) (SARM:2)

Covance Laboratories Inc.

1. Bacteriological Analytical Manual, Salmonella, Chapter 5, 8th Edition, 2006. Food and Drug Administration, AOAC International: Gaithersburg, Maryland. Modified.
2. Compendium of Methods for the Microbiological Examination of Foods, Salmonella, Chapter 37, 4th Edition, 2001. American Public Health Association. Washington D.C. Modified.
3. Salmonella in Foods, AOAC Official Method 990.13, DNA hybridization Method. Official Methods of Analysis of the AOAC International, 18th Edition, 2005. Gaithersburg, Maryland. Modified.

#### Selenium (SEHG\_S:4)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 986.15, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

#### Thiamin (BIDE\_S:6)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 942.23, 953.17, and 957.17, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

#### Vitamin A (AFD1\_S:4)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 974.29, 992.04, and 992.06, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

Thompson, J.N., and Duval, S., "Determination of Vitamin A in Milk and Infant Formula by HPLC", Journal of Micronutrient Analysis, 6:147-159, (1989).

#### Vitamin B12 (B12F\_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 952.20 and 960.46, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005).

The United States Pharmacopeia, 29th Ed., pp. 603-4, United States Pharmacopeial Convention, Inc.: Rockville, Maryland (2005).

Methods of Analysis for Infant Formulas, Infant Formula Council, Atlanta, Georgia, Section C-2, (1985).

#### Vitamin D (DFD1\_S:7)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 982.29.

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### Method References

### Testing Location

#### Vitamin E (EFD1\_S:3)

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Cort, W. M., Vicente, T. S., Waysek, E. H., and Williams, B. D., Journal of Agricultural Food Chemistry, 31:1330-1333 (1983). (Modified)

Speek, A. J., Schijver, J., and Schreurs, W. H. P., Journal of Food Science, 50:121-124 (1985). (Modified)

McMurray, C. H., Blanchflower, W. J., and Rice, D. A., Journal of the Association of Official Analytical Chemists, 63: 1258-1261 (1980).

#### Yeast and Mold Count (YMCM:4)

Covance Laboratories Inc.

1. Bacteriological Analytical Manual, Yeasts, Molds and Mycotoxins. Chapter 18, 8th Edition, 2001. Food and Drug Administration, AOAC International: Gaithersburg, Maryland. Modified.

2. Compendium of Methods for the Microbiological Examination of Foods, Yeasts and Molds, Chapter 20, 4th Edition, 2001. American Public Health Association, Washington D.C. Modified.

3. Yeast and Mold Counts in Foods, AOAC Official Method 997.02. Dry Rehydratable Film Method (Petrifilm). Official Methods of Analysis of the AOAC International, 18th Edition, 2005. Gaithersburg, Maryland. Modified.

### Testing Location(s)

**Covance Laboratories Inc.**  
3301 Kinsman Blvd  
Madison WI 53704  
608-241-4471

**These results apply only to the items tested. This certificate of analysis shall not be reproduced, except in its entirety, without the written approval of Covance.**