

## MIX 10 (<sup>13</sup>C Fusarium toxins)

### 1. General information

This document is designed and the certified value(s) and uncertainty(ies) are determined in accordance with ISO Guide 31 [1] and Eurachem / CITAC Guides [2,3].

### 2. Description of the Reference Material (RM)

Name:	Mix 10 ( <sup>13</sup> C Fusarium toxins)
Catalog number:	B-MYC0913-1.2
Lot #:	I17011A
Certificate version:	1
Expiry date:	04.07.2018
Starting materials:	U-[ <sup>13</sup> C <sub>15</sub> ]-Deoxynivalenol, Lot # IS14472D, U-[ <sup>13</sup> C <sub>22</sub> ]-HT-2 Toxin, Lot # IS14472H, U-[ <sup>13</sup> C <sub>24</sub> ]-T-2 Toxin, Lot # IS14472T, U-[ <sup>13</sup> C <sub>18</sub> ]-Zearalenone, Lot # IS12471Z Romer Labs Diagnostic GmbH
Matrix:	Acetonitrile, LiChrosolv Lot # I796830537, Merck, Catalog Nr. 1000305000
Physical description of RM:	<sup>13</sup> C Fusarium toxins in Acetonitrile
Packaging and amount of RM:	Amber Certan® glass ampoules, solution of 1.2 mL
Name and address of the manufacturer:	Romer Labs Diagnostic GmbH Technopark 1, 3430 Tulln, Austria
Name and address of the supplier:	LGC Standards GmbH Mercatorstraße 51, 46485 Wesel, Germany Tel +49(0)2 81 98 87 0, Fax +49(0)2 81/98 87 199 www.lgcstandards.com

#### 2.1 Intended use of the RM

- for laboratory use only
- internal standard [4, 5]

#### 2.2 Instruction for the correct use of the RM

The ampoules should be stored at -18 to -22°C in a dark place. Before usage of the RM, the ampoules should be allowed to warm to room temperature. The recommended minimum sub-sample amount for all kinds of application is 100 µL. The expiry date of this RM is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/packages.

#### 2.3 Hazardous situation

The normal laboratory safety precautions should be observed when working with this RM. Further details for the handling of this RM are available as safety data sheet.

Hazardous Ingredients	Concentration in %	Pictograms	Signal word	Hazard statement(s)
Acetonitrile	> 99.9		Danger	H225, H302, H312, H319, H332

### 3. Certified values and their uncertainties

Mix 10 ( <sup>13</sup> C Fusarium toxins)		
Compound	Mass concentration <sup>a</sup>	
	Certified value <sup>b</sup>	Uncertainty <sup>c</sup>
U-[ <sup>13</sup> C <sub>15</sub> ]-Deoxynivalenol, 99.0 atom % <sup>13</sup> C	10.0 µg/mL	± 0.10 µg/mL
U-[ <sup>13</sup> C <sub>22</sub> ]-HT-2 Toxin, 99.5 atom % <sup>13</sup> C	10.1 µg/mL	± 0.26 µg/mL
U-[ <sup>13</sup> C <sub>24</sub> ]-T-2 Toxin, 99.3 atom % <sup>13</sup> C	1.02 µg/mL	± 0.02 µg/mL
U-[ <sup>13</sup> C <sub>18</sub> ]-Zearalenone, 99.5 atom % <sup>13</sup> C	3.02 µg/mL	± 0.05 µg/mL

<sup>a</sup> Values are based on preparation data and confirmed experimentally by HPLC-UV and HPLC-MS/MS analysis  
<sup>b</sup> Mass concentration based on weighed amount, purity and dilution step  
<sup>c</sup> Expanded uncertainty U (k = 2) of the value u<sub>c</sub> according to GUM [6]

#### 3.1 Calculation of uncertainty

The uncertainty of each certified value was calculated on the basis of preparation [7].

Uncertainty components	Description	Standard uncertainty (u)	
Purity (P) of solid U-[ <sup>13</sup> C <sub>15</sub> ]-Deoxynivalenol, 99.0 atom % <sup>13</sup> C U-[ <sup>13</sup> C <sub>22</sub> ]-HT-2 Toxin, 99.5 atom % <sup>13</sup> C U-[ <sup>13</sup> C <sub>24</sub> ]-T-2 Toxin, 99.3 atom % <sup>13</sup> C U-[ <sup>13</sup> C <sub>18</sub> ]-Zearalenone, 99.5 atom % <sup>13</sup> C	P <sub>1</sub> = 99.5 ± 0.5 % P <sub>2</sub> = 98.0 ± 2.0 % P <sub>3</sub> = 98.8 ± 1.2 % P <sub>4</sub> = 98.7 ± 1.3 %	<b>u (P<sub>1</sub>) = 0.3 %</b> <b>u (P<sub>2</sub>) = 1.2 %</b> <b>u (P<sub>3</sub>) = 0.7 %</b> <b>u (P<sub>4</sub>) = 0.8 %</b>	a
Weighing procedure weighted sample: m <sub>ws(13C DON)</sub> = 10.052 mg m <sub>ws(13C HT-2)</sub> = 10.274 mg m <sub>ws(13C T-2)</sub> = 1.030 mg m <sub>ws(13C ZON)</sub> = 3.060 mg	U(m) = 0.0000008g + 1.30 * 10 <sup>-5</sup> * m <sub>Toxin</sub> u(m) = U(m)/2	<b>u (m) = 0.0005 mg</b>	b
Dilution procedure volumetric flask: V <sub>11</sub> = 100 mL volumetric flask 2: V <sub>12</sub> = 250 mL one-mark glass pipette: V <sub>p</sub> = 25 mL	calibration flask 1: 100 mL ± 0.1 mL repeatability flask 1: 0.04 mL volume expansion solvent flask 1  calibration flask 2: 250 mL ± 0.15 mL repeatability flask 2: 0.03 mL volume expansion solvent flask 2  calibration pipette: 25 mL ± 0.03 mL volume expansion solvent pipette	u (cal1) = 0.04 mL u (rep1) = 0.04 mL u (Vol. exp.1) = 0.24 mL <b>u (V1) = 0.3 mL</b> u (cal2) = 0.06 mL u (rep2) = 0.03 mL u (Vol. exp.2) = 0.59 mL <b>u (V2) = 0.59 mL</b> u (cal3) = 0.01 mL u (Vol. exp.3) = 0.06 mL <b>u (V3) = 0.06 mL</b>	c d e f g h i j k l m

<sup>a</sup> Maximum tolerance of purity (rectangular distribution) was divided by  $\sqrt{3}$   
<sup>b</sup> Calculation of this u-value is based upon the uncertainty formula for the weighed amount as given in the calibration report from annual balance calibration  
<sup>c,g,k</sup> A triangular distribution (division by  $\sqrt{6}$ ) was chosen for the calculation of u (cal)  
<sup>d,h</sup> Based on a series of ten fill and weigh experiments on a typical 100 mL flask and a 250 mL flask; the value was used directly as a standard deviation  
<sup>e,l</sup> Estimation based on the density of pure acetonitrile = 0.7857 g/cm<sup>3</sup> at temperature T = 20°C and a maximum temperature variation of ± 3°C of volume expansion, relative volume expansion coefficient of acetonitrile is 1370 \* 10<sup>-6</sup>/°C [8], volume expansion term (rectangular distribution) was divided by  $\sqrt{3}$   
<sup>f,j,m</sup> The three contributions are combined to give the u (V) =  $\sqrt{u(\text{cal})^2 + u(\text{rep})^2 + u(\text{Vol. exp.})^2}$

**Calculation of the combined uncertainty  $u_c$  and the expanded standard uncertainty  $U$  for  $^{13}\text{C}$ -Deoxynivalenol as example**

$$c_{\text{Toxin}} = \frac{10 \times m_{\text{ws}^{13}\text{C DON}} \times P_1 \times V_p}{V_{f1} \times V_{f2}} = \frac{10 \times 10.052 \times 99.5 \times 25}{100 \times 250} = 10.0 \text{ mg / L}$$

$$\frac{u_c(c_{\text{Toxin}})}{c_{\text{Toxin}}} = \sqrt{\left[\frac{u(P_1)}{P_1}\right]^2 + \left[\frac{u(m)}{m_{\text{ws}^{13}\text{C DON}}}\right]^2 + \left[\frac{u(V1)}{V_{f1}}\right]^2 + \left[\frac{u(V2)}{V_{f2}}\right]^2 + \left[\frac{u(V3)}{V_p}\right]^2} = \sqrt{\left[\frac{0.3}{99.5}\right]^2 + \left[\frac{0.0005}{10.052}\right]^2 + \left[\frac{0.3}{100}\right]^2 + \left[\frac{0.59}{250}\right]^2 + \left[\frac{0.06}{25}\right]^2} = 0.005$$

$$u_c(c_{\text{Toxin}}) = c_{\text{Toxin}} \times 0.005 = 10.0 \times 0.005 = 0.05 \text{ mg / L}$$

Calculation of expanded standard uncertainty  $U$  using a coverage factor  $k = 2$

$$U(c_{\text{Toxin}}) = u_c(c_{\text{Toxin}}) \times 2 = 0.05 \times 2 = 0.10 \text{ mg / L} = 0.10 \mu\text{g / mL}$$

## 4. Isotopic enrichment and isotope pattern

Isotope pattern							
Compound	Isotopic distribution						
[ $^{13}\text{C}_{15}$ ]-Deoxynivalenol	86.5 %	[ $^{13}\text{C}_{22}$ ]-HT-2 Toxin	89.7 %	[ $^{13}\text{C}_{24}$ ]-T-2 Toxin	84.5 %	[ $^{13}\text{C}_{18}$ ]-Zearalenone	91.0 %
[ $^{13}\text{C}_{14}$ ]-Deoxynivalenol	11.5 %	[ $^{13}\text{C}_{21}$ ]-HT-2 Toxin	9.0 %	[ $^{13}\text{C}_{23}$ ]-T-2 Toxin	13.7 %	[ $^{13}\text{C}_{17}$ ]-Zearalenone	8.5 %
[ $^{13}\text{C}_{13}$ ]-Deoxynivalenol	2.1 %	[ $^{13}\text{C}_{20}$ ]-HT-2 Toxin	1.3 %	[ $^{13}\text{C}_{22}$ ]-T-2 Toxin	1.7 %	[ $^{13}\text{C}_{16}$ ]-Zearalenone	0.5 %
<b>Calculated isotopic enrichment level<sup>a</sup> : 99.0 atom % <math>^{13}\text{C}</math></b>		<b>Calculated isotopic enrichment level<sup>a</sup> : 99.5 atom % <math>^{13}\text{C}</math></b>		<b>Calculated isotopic enrichment level<sup>a</sup> : 99.3 atom % <math>^{13}\text{C}</math></b>		<b>Calculated isotopic enrichment level<sup>a</sup> : 99.5 atom % <math>^{13}\text{C}</math></b>	

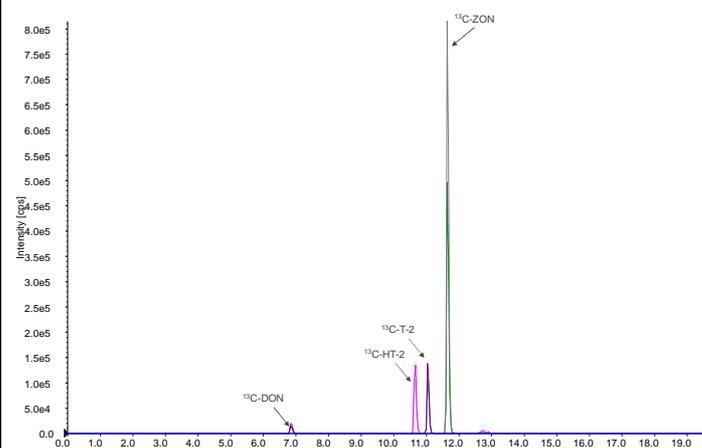
<sup>a</sup> Approximation based on LC-MS data

## 5. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [7]. Thus the certified values (mass concentration of  $^{13}\text{C}$  Fusarium toxins) are based on the weighed amount of the starting materials and are therefore traceable to the stated purity of the solid raw materials. High purity material represents a practical realization of concentration units, through conversion of mass to molar quantity.

## 6. Confirmation of certified value by LC-MS/MS [9]:

The certified concentration value of <sup>13</sup>C Fusarium toxins of the gravimetric prepared solution was confirmed by LC-UV and LC-MS/MS against an independently prepared reference batch.

column	Phenomenex Gemini® C <sub>18</sub> -column, 150 x 4.6 mm, 5µm																											
sample preparation	diluted 1:60 and 1:20 with solvent A																											
injection volume	10 µL																											
solvent A	methanol/water/acetic acid 10/89/1 with 5mM ammonium acetate																											
solvent B	methanol/water/acetic acid 97/2/1 with 5mM ammonium acetate																											
flow rate	1.0 mL / min																											
gradient	time in minutes (min)	% solvent B																										
	0 – 2	0																										
	2 – 10.1	0-100																										
	10.1 – 14.0	100																										
	14.0 – 15.5	100 - 0																										
	15.5 – 19.5	100																										
source type	ESI		<p>Figure 1: XICs of <sup>13</sup>C-DON, <sup>13</sup>C-HT-2, <sup>13</sup>C-T-2 and <sup>13</sup>C-ZON in + and - MRM (Mix10_I17011A_20_1)</p> <table border="1"> <thead> <tr> <th>Analyte</th> <th>Precursor ion (m/z)</th> <th>Product ion (m/z)</th> <th>Retention Time (min)</th> <th>Concentration<sup>a</sup> (µg/mL)</th> </tr> </thead> <tbody> <tr> <td><sup>13</sup>C-DON</td> <td>370.1</td> <td>59.1</td> <td>6.84</td> <td>9.92 ± 0.3</td> </tr> <tr> <td><sup>13</sup>C-HT-2</td> <td>464.3</td> <td>113.0</td> <td>10.66</td> <td>9.73 ± 0.3</td> </tr> <tr> <td><sup>13</sup>C-T-2</td> <td>508.2</td> <td>229.1</td> <td>11.31</td> <td>1.01 ± 0.01</td> </tr> <tr> <td><sup>13</sup>C-ZON</td> <td>335.1</td> <td>185.0</td> <td>11.64</td> <td>3.04 ± 0.03</td> </tr> </tbody> </table> <p><sup>a</sup> Mean of 6 replicate measurements against reference batch, confidence interval with P = 95 %</p>	Analyte	Precursor ion (m/z)	Product ion (m/z)	Retention Time (min)	Concentration <sup>a</sup> (µg/mL)	<sup>13</sup> C-DON	370.1	59.1	6.84	9.92 ± 0.3	<sup>13</sup> C-HT-2	464.3	113.0	10.66	9.73 ± 0.3	<sup>13</sup> C-T-2	508.2	229.1	11.31	1.01 ± 0.01	<sup>13</sup> C-ZON	335.1	185.0	11.64	3.04 ± 0.03
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scan type	MRM scans in positive (for <sup>13</sup> C-HT-2 and <sup>13</sup> C-T-2) and negative ionization mode (for <sup>13</sup> C-DON and <sup>13</sup> C-ZON)																											

## 7. Further information

The purchaser must determine the suitability of this product for its particular use. LGC Standards GmbH makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by LGC Standards GmbH. We do not guarantee that the product can be used for a special application.

approved for release by: Dipl. Chem. Ulrich Berger

date: 04.01.2017

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### References:

- [1] ISO Guide 31, 1-7, (2000), "Reference materials – Contents of certificates and labels"
- [2] Eurachem / CITAC Guide CG-4, 1-133, (2012), "Quantifying Uncertainty in Analytical Measurement"
- [3] Eurachem / CITAC Guide CG4, 1-133, (QUAM:2012.P1), "Quantifying Uncertainty in Analytical Measurement", 3<sup>rd</sup> Ed.
- [4] G. Häubl, F. Berthiller, R. Krska, R. Schuhmacher, "Suitability of a fully <sup>13</sup>C isotope labelled internal standard for the determination of the mycotoxin deoxynivalenol by LC-MS/MS without clean-up", Anal. Bioanal. Chem. **384** (3), (2006), 692-696
- [5] G. Häubl, F. Berthiller, J. Rechthaler, G. Jaunecker, E.M. Binder, R. Krska, R. Schuhmacher, (2006), "Characterization and application of isotope-substituted (<sup>13</sup>C<sub>15</sub>)-deoxynivalenol (DON) as an internal standard for the determination of DON", Food Addit Contam. **23**, (2006), 1187-1193
- [6] International Organization for Standardization (ISO), (2008), "Guide to the expression of uncertainty in measurement", (GUM 1995 with minor corrections) 1<sup>st</sup> Ed. Geneva, Switzerland
- [7] R.D. Josephs, R. Krska, S. MacDonald, P. Wilson, H. Pettersson, J. AOAC Int. **86**, 50-60, (2003), "Preparation of a Calibrant as Certified Reference Material for Determination of the Fusarium Mycotoxin Zearalenone"
- [8] E.W. Flick, (1998), "Industrial Solvents Handbook", 5<sup>th</sup> Ed., Noyes Data Corp. Westwood NJ
- [9] F. Berthiller, R. Schuhmacher, G. Buttlinger, R. Krska, Journal of Chromatography A **1062**, 209-216, (2005), "Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography – tandem mass spectrometry"