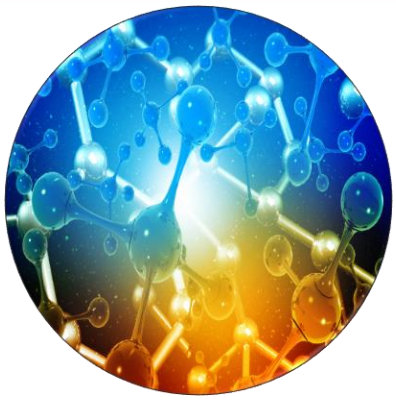


Ph. Eur. Monograph 2780: Temozolomide Related Substances on Luna™ 5µm C18(2) and Luna 5 µm C18 Columns

Janja Vasilić[1], Amra Perva Uzunalić, PhD[1], Dirk Hansen, PhD[3], Bryan Tackett, PhD[2], and Heiko Behr, PhD[3]
[1]University of Maribor, Faculty of Chemistry and Chemical Engineering Group for Separation Analysis, Smetanova 17, 2000 Maribor, Slovenia
[2]Phenomenex, Inc. 411 Madrid Ave., Torrance, CA 90501 USA
[3]Phenomenex Ltd. Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany

Overview

Temolozomide is a cytostatic drug used for the treatment of brain tumors like glioblastoma and anaplastic astrocytoma. The drug can be administered either orally or intravenously.

In this application note we show the separation of Temozolomide from its related substances following Ph. Eur. Monograph 2780. We used a Luna 5 µm C18(2) 150 x 4.6 mm column and a Luna 5 µm C18 150 x 4.6 mm column for the study.

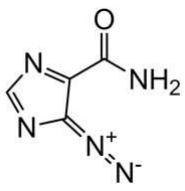
Both the Luna C18(2) and the Luna C18 column met the system suitability criteria for Related Substances analysis of a resolution (R_S) minimum of 1.5 between the peaks due to impurity B and Temozolomide in the chromatogram obtained with Reference Solution (b).

During the study we observed a fast degradation of Impurity D (4-Diazo-4*H*-imidazole-5-carboxamide) in Reference Solution (c). Based on this we recommend the use of freshly prepared reference solution for the peak identification of Impurity D.

The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM)–Council of Europe; Postal address: Allee Kastner CS 30026 F-67081 Strasbourg (France):

- Y0001827, Temozolomide CRS, batch 1
- Y0001960, Temozolomide for peak identification CRS, batch 1
- Y0000734, Dacarbazine impurity A CRS, batch 3 (corresponds to Temozolomide impurity E)

Figure 1. Impurity D Structure



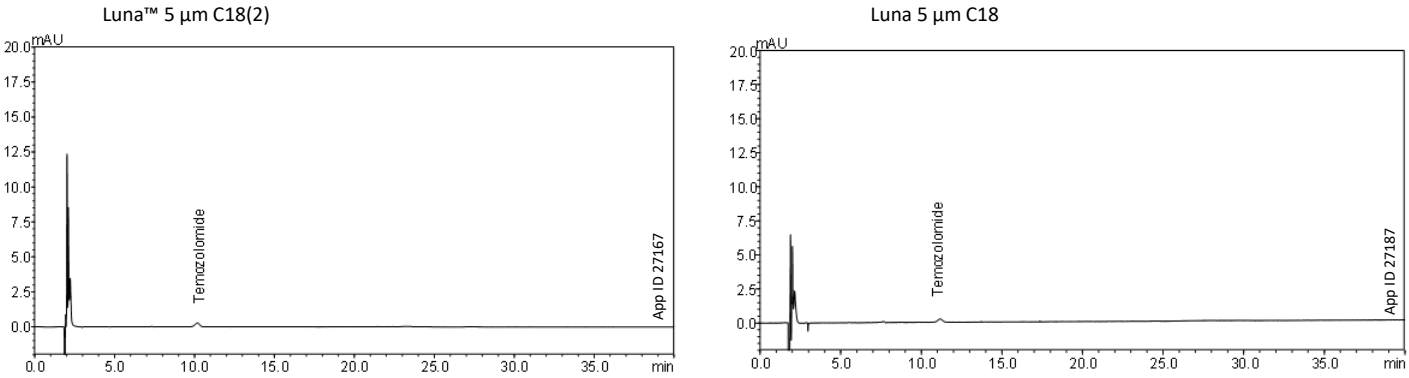
LC-UV Conditions

Columns: Luna 5 µm C18(2) (00F-4252-E0)
Luna 5 µm C18 (00F-4041-E0)
Dimensions: 150 x 4.6 mm
Mobile Phase: Mobile Phase (**Table 1**)
Flow Rate: 1.0 mL/min
Injection: 10 µL
Temperature: 25 °C
Detector: UV @ 270 nm
System: Agilent® 1260 Infinity II

Table 1. Preparation of Test and Reference Solutions

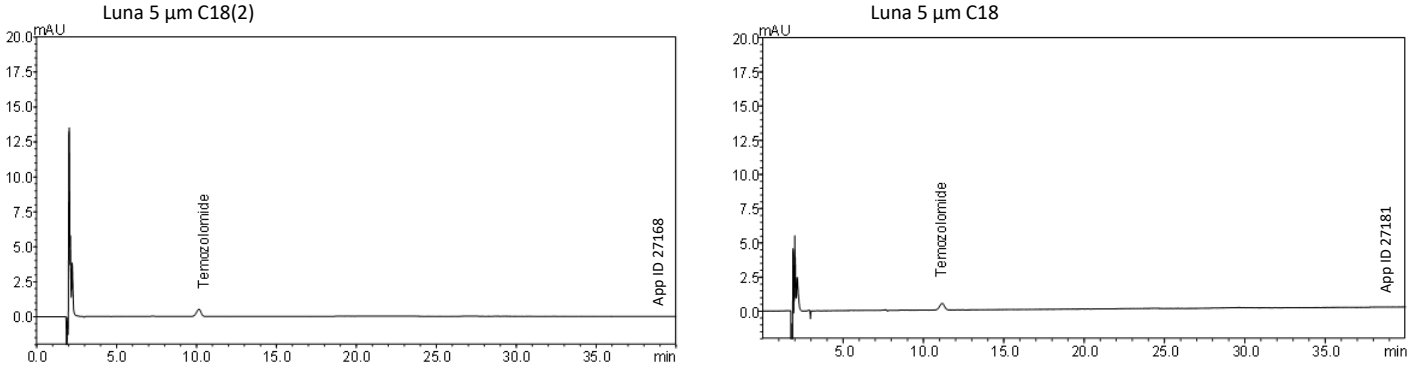
Solution	Composition
Mobile Phase	Dissolve 0.94 g of Sodium Hexane-1-sulfonate in 1 L of a mixture of Methanol / 0.5 % V/V acetic acid solution (4:96, V/V).
Test Solution (SaS)	Dissolve approx. 25.0 mg reference substance Temozolomide in Dimethyl Sulfoxide and dilute to 25.0 mL with the same solvent. Mix well (SaS).
Reference Solution (a)	1. Transfer 1.0 mL of Test Solution (SaS) into a 100 mL volumetric flask, fill up to volume with Dimethyl Sulfoxide and mix well (RSa-1). 2. Transfer 1.0 mL of RSa-1 into a 10 mL volumetric flask, fill up to volume with Dimethyl Sulfoxide and mix well (**RSa**).
Solution for System Sensitivity (SSens)	Transfer 1.0 mL of RSa-1 into a 20 mL volumetric flask, fill up to volume with Dimethyl Sulfoxide and mix well (**SSens**). The solution is prepared at the concentration of reporting threshold (0.05 %).
Reference Solution (b)	In order to prepare Temozolamide impurities A, B and E in situ, mix 5 mL of Hydrochloric Acid solution with 5 mL of Test Solution in a volumetric flask. Heat the mixture in a water bath for 1 h (up to boiling point temperature) (**RSb**).
Reference Solution (c)	Transfer 2 mg of Temozolomide for peak identification (containing impurity D) into 2 mL flask and dilute with Dimethyl Sulfoxide (**RSc**).

Figure 2. System Sensitivity Test



Solution for system sensitivity (reporting threshold of 0.05 % achieved: S/N > 10)

Figure 3. Reference Solution (a)

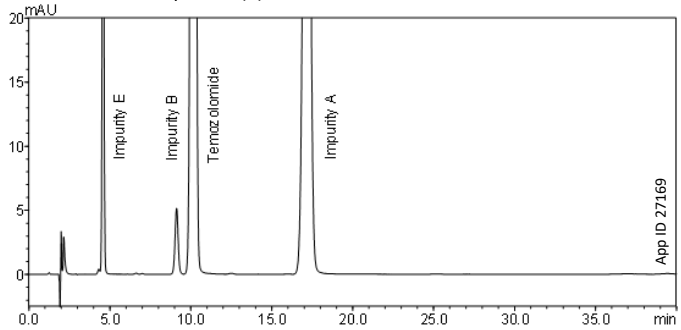


Inj. No.	t_R (min)	Area	Symmetry Factor
1	10.194	10179	0.89
2	10.185	10125	0.89
3	10.164	10205	0.92
4	10.171	10147	0.88
5	10.163	10218	0.88
6	10.155	10041	0.91
Average	10.172	10152	0.90
%RSD	0.15	0.64	1.9

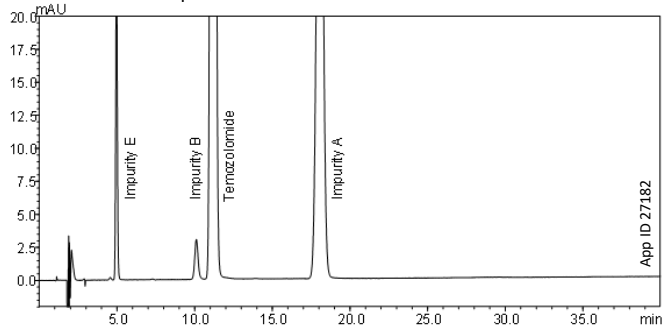
Inj. No.	t_R (min)	Area	Symmetry Factor
1	11.173	9909	0.93
2	11.129	10144	0.99
3	11.150	9627	1.04
4	11.170	10044	1.00
5	11.178	9529	0.91
6	11.152	9791	1.00
Average	11.159	9841	0.98
%RSD	0.17	2.42	4.98

Figure 4. System Suitability Test Using Reference Solution (b)

Luna™ 5 µm C18(2)



Luna 5 µm C18

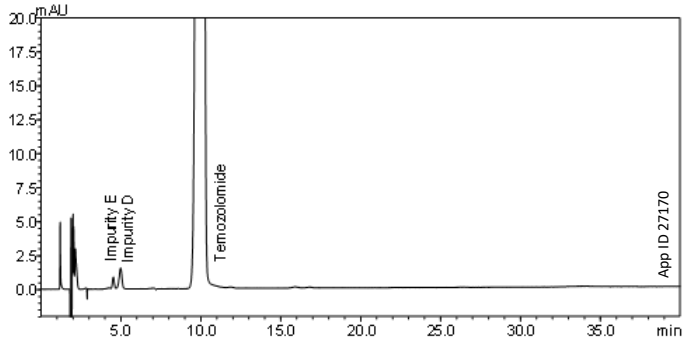


Inj. No.	Imp. E	Imp. B	Imp. A	Temozolomide		RS
	t_R(min) Area	t_R(min) Area	t_R(min) Area	t_R(min) Area	Symmetry Factor	SST Ref. (b)
1	4.572	9.140	17.159	10.168	1.02	2.69
	408448	69679	1977146	4018037		
2	4.573	9.135	17.158	10.162	1.02	2.69
	408464	69679	1980955	4017238		
3	4.574	9.131	17.158	10.158	1.02	2.69
	408085	69640	1982547	4012783		
4	4.575	9.128	17.160	10.154	1.02	2.69
	408710	69550	1984647	4010530		
5	4.576	9.124	17.161	10.149	1.02	2.69
	408389	69579	1990514	4011604		
6	4.577	9.121	17.168	10.147	1.02	2.69
	408657	69590	1994781	4011404		
Avg.	4.574	9.130	17.161	10.156	1.02	2.69
	408459	69620	1985098	4013599		
%RSD	0.039	0.080	0.021	0.080	0.025	0.034
	0.054	0.078	0.33	0.080		

Inj. No.	Imp. E	Imp. B	Imp. A	Temozolomide		RS
	t_R(min) Area	t_R(min) Area	t_R(min) Area	t_R(min) Area	Symmetry Factor	SST Ref. (b)
1	4.966	10.111	18.058	11.173	1.08	2.52
	193595	45308	1395766	4416035		
2	4.967	10.108	18.060	11.170	1.08	2.52
	193565	45680	1398477	4414969		
3	4.968	10.104	18.063	11.167	1.08	2.53
	193445	45600	1401967	4415686		
4	4.968	10.101	18.067	11.164	1.08	2.53
	193551	45611	1406614	4417003		
5	4.968	10.098	18.070	11,162	1.08	2.53
	4193792	45864	1409025	4407466		
6	4.969	10.096	18.072	11.159	1.08	2.53
	193264	45448	1408899	4399177		
Avg.	4.968	10.103	18.065	11.166	1.08	2.53
	193535	45585	1403458	4411723		
%RSD	0.022	0.056	0.03	0.045	0.12	0.23
	0.09	0.042	0.40	0.16		

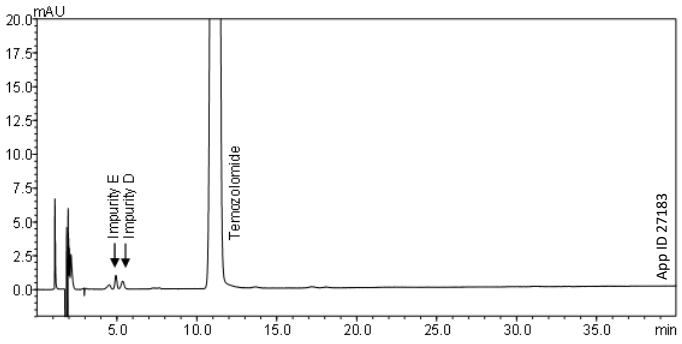
Figure 5. Peak Identification of Impurity D using Reference Solution (c)

Luna™ 5 µm C18(2)



Temozolomide		Impurity E		Impurity D	
t_R (min)	Area	t_R (min)	Area	t_R (min)	Area
9.847	9648267	4.530	7280	4.964	12619

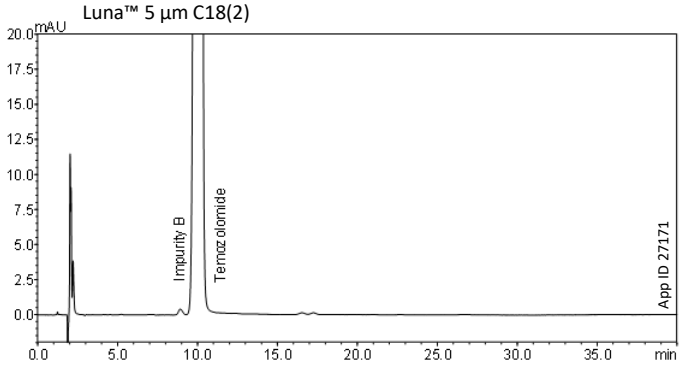
Luna 5 µm C18



Temozolomide		Impurity E		Impurity D	
t_R (min)	Area	t_R (min)	Area	t_R (min)	Area
11.134	10759343	4.938	8847	5.364	7286

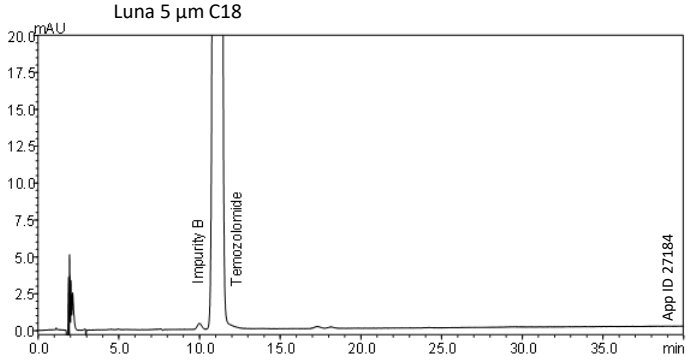
Figure 6. Test Solution (SaS)

Luna™ 5 µm C18(2)



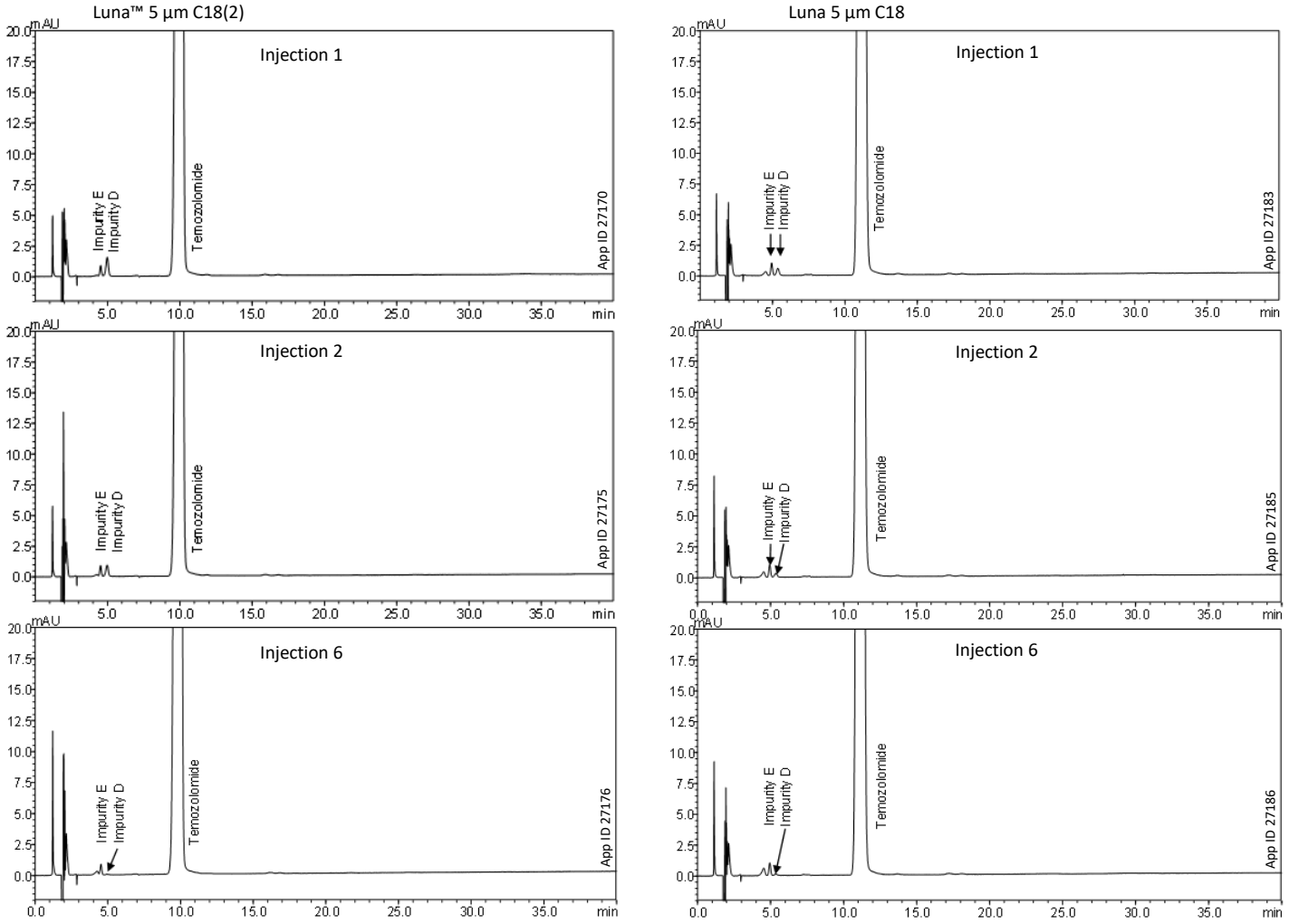
Inj. No.	Impurity B		Temozolomide			RS Imp. B / Temozolomide
	t_R (min)	Area	t_R (min)	Area	Symmetry Factor	
1	8.949	7151	10.062	10183768	0.97	2.21
2	8.953	7083	10.059	10182114	0.97	2.20
3	8.948	7104	10.055	10181689	0.97	2.20
4	8.945	7151	10.048	10171473	0.97	2.19
5	8.930	6891	10.044	10052506	0.96	2.21
6	8.923	7177	10.041	10179088	0.97	2.23
Avg.	8.941	7093	10.052	10158440	0.97	2.21
%RSD	0.13	1.5	0.087	0.51	0.35	0.56

Luna 5 µm C18



Inj. No.	Impurity B		Temozolomide			RS Imp. B / Temozolomide
	t_R (min)	Area	t_R (min)	Area	Symmetry Factor	
1	9.970	7710	11.101	10180015	1.016	2.086
2	9.971	7452	11.098	10188935	1.011	2.107
3	9.988	7607	11.095	10191840	1.011	2.025
4	9.960	7472	11.092	10181738	1.010	2.088
5	9.986	7507	11.089	10182425	1.009	2.060
6	9.973	7425	11.085	10201193	1.009	2.068
Avg.	9.975	7529	11.093	10187691	1.011	2.072
%RSD	0.11	1.45	0.052	0.079	0.25	1.37

Figure 7. Degradation of Impurity D in Solution



No.	Impurity E		Impurity D		Temozolomide	
	t_R (min)	Area	t_R (min)	Area	t_R (min)	Area
1	4.530	7280	4.964	12619	9.847	9648267
2	4.529	7115	4.971	2815	9.843	9646242
3	4.527	7168	4.965	2050	9.842	9628177
4	4.528	6957	4.938	825	9.840	9634707
5	4.530	7163	4.937	806	9.839	9637224
6	4.531	6937	4.917	487	9.839	9637095
Avg.	4.529	7103	4.948	3267	9.841	9638619
%RSD	0.036	1.87	0.42	142.85	0.032	0.078

No.	Impurity E		Impurity D		Temozolomide	
	t_R (min)	Area	t_R (min)	Area	t_R (min)	Area
1	4.938	8847	5.364	7286	11.134	10759343
2	4.940	8836	5.362	4060	11.131	10773640
3	4.941	8849	5.372	2959	11.128	10768611
4	4.941	8759	5.372	2332	11.128	10756753
5	4.942	8815	5.361	1883	11.127	10761384
6	4.945	8746	5.372	1228	11.125	10761475
Avg.	4.941	8809	5.367	3291	11.129	10763534
%RSD	0.045	0.52	0.10	66.31	0.028	0.059

Conclusion

Both columns used in this study met the system requirements of Ph. Eur. Monograph 2780 describing the analysis of Temozolomide and related substances. Due to the instability of Impurity D in solution it is critical to prepare Reference Solution (c) fresh before each sequence. After 5 h no Impurity D could be detected in the preparation of Reference Solution (c).

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com