MRMplus Retention Time Marker (#FMR-002)

MRMplus Retention Time Marker is the mixture of marker peptides (12 synthetic peptides) for calculating the retention times of LC in LC-MS/MS system for scheduled-MRM. The segment value of each MRMplus Standard Peptide is easily converted by using this marker and the conversion software.

For research use only. Not for use in diagnostic procedures.
Avoid freeze-thaw cycles.

Component

200 x MRMplus Retention Time Marker Stock Solution (12 µl)

Dilute 1 µl of **Stock Solution** into 199 µl of sample diluent for your LC system as Working Solution. When you use TFA as the sample diluent, grass ampule type product (eg. #28902 from Thermo Scientific) is recommended. Working Solution should be stored at 4ºC
NOTE: Please use working solution within **ONE WEEK**

Storage

-80ºC

General Protocol

This protocol is optimized for QTRAP5500 System (nanoESI, ABSciex). For the other LC-MS/MS systems, optimal condition should be determined by users.

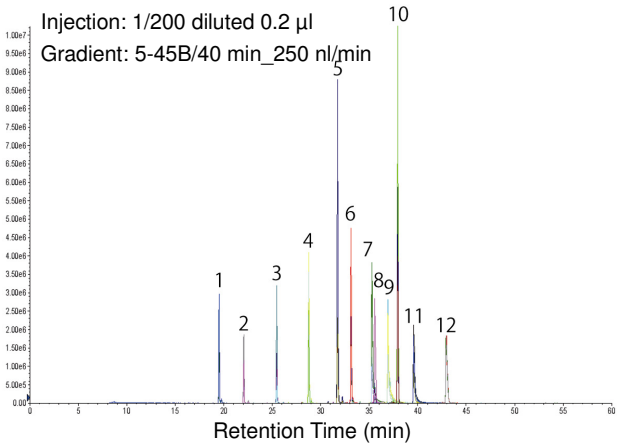
(1) Inject 0.2 µl of the Working Solution into LC.
(2) Optimize LC conditions and injection volume.

NOTE: Initial guidline for LC conditions: 5-40%B/40min @ 200nl for 100 µm in inside diameter, 16 cm long LC column. You should optimize conditions by using this **Retention Time Marker** whenever you change LC column. For example, try 35% to 50% as soluvent B, and 180 to 300 nl/min. Some LC-MS/MS instruments may use 5-45%B for initial condition.

(3) Input the values of Q1 and Q3 for 12 marker peptides and conduct MRM analysis without scheduling function.
(4) Write down the detected retention time of 12 marker peptides into spread sheet.
(5) Convert segment values of internal standard peptides specific to your target proteins into expected retention time using the detected retention time and conversion software (RTimeToSegment.exe).
(6) Input the expected retention time into your LC-MS/MS system.
(7) Mix 1 µl **Retention Time Marker** for 1 µg sample, and then add the suitable amount of internal standard peptide. Dilute the mixture to 10 µl with sample diluent, use 2 µl for conduct scheduled MRM analysis.

NOTE: You can find the information of the Q1, Q3 values, and conversion software (RTimeToSegment.exe) on FUNAKOSHI website（http://www.funakoshi.co.jp/contents/7979）.
NOTE: You should set a wide range of expected retention time for scheduling, or you would miss the target peptide.
NOTE: Some marker peptides may appear closely each other or sometimes interchanged depending on your LC conditions, such as gradient, colums used, or room temperature.

MRM Settings of MRMplus Retention Time Marker

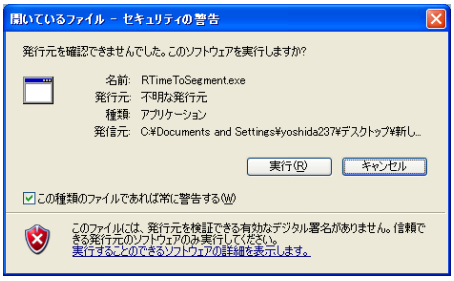


Marker#	Q1	Q3	RTime	ID	DP	EP	CE	CXP
1	484.26	696.40		RTM1.peptide	50	10	26.31	26.35
	484.26	599.35		RTM1.peptide	50	10	26.31	22.85
	484.26	528.31		RTM1.peptide	50	10	26.31	20.28
2	590.82	599.35		RTM2.peptide	50	10	31.00	22.85
	590.82	909.52		RTM2.peptide	50	10	31.00	34.04
	590.82	741.43		RTM2.peptide	50	10	31.00	27.98
3	432.25	651.35		RTM3.peptide	50	10	24.02	24.72
	432.25	750.41		RTM3.peptide	50	10	24.02	28.30
	432.25	523.29		RTM3.peptide	50	10	24.02	20.10
4	601.84	817.48		RTM4.peptide	50	10	31.48	30.72
	601.84	718.41		RTM4.peptide	50	10	31.48	27.14
	601.84	605.33		RTM4.peptide	50	10	31.48	23.06
5	489.78	779.47		RTM5.peptide	50	10	26.55	29.35
	489.78	531.35		RTM5.peptide	50	10	26.55	20.39
	489.78	678.42		RTM5.peptide	50	10	26.55	25.70
6	528.27	885.44		RTM6.peptide	50	10	28.24	33.17
	528.27	784.39		RTM6.peptide	50	10	28.24	29.53
	528.27	637.32		RTM6.peptide	50	10	28.24	24.22
7	607.82	938.52		RTM7.peptide	50	10	31.74	35.09
	607.82	722.44		RTM7.peptide	50	10	31.74	27.29
	607.82	623.38		RTM7.peptide	50	10	31.74	23.71
8	503.96	728.39		RTM8.peptide	50	10	29.20	27.51
	503.96	631.34		RTM8.peptide	50	10	29.20	24.00
	503.96	516.31		RTM8.peptide	50	10	29.20	19.85
9	675.85	821.45		RTM9.peptide	50	10	34.74	30.86
	675.85	607.32		RTM9.peptide	50	10	34.74	23.13
	675.85	720.40		RTM9.peptide	50	10	34.74	27.22
10	530.31	762.41		RTM10.peptide	50	10	28.33	28.73
	530.31	691.38		RTM10.peptide	50	10	28.33	26.17
	530.31	875.50		RTM10.peptide	50	10	28.33	32.82
11	781.92	937.50		RTM11.peptide	50	10	39.40	35.05
	781.92	562.32		RTM11.peptide	50	10	39.40	21.51
	781.92	709.39		RTM11.peptide	50	10	39.40	26.82
12	661.38	906.51		RTM12.peptide	50	10	37.07	33.94
	661.38	836.43		RTM12.peptide	50	10	37.07	31.41
	661.38	502.76		RTM12.peptide	50	10	37.07	19.36

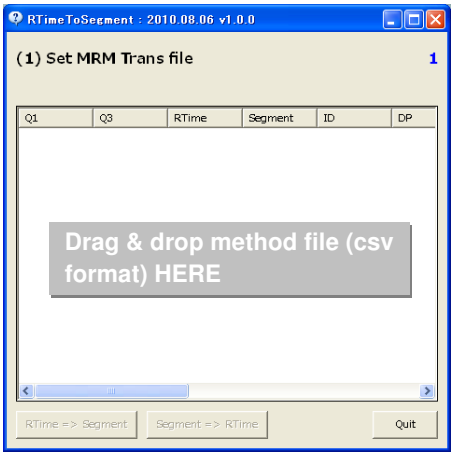
How to use conversion software

(1) Start up RTimeToSegment.exe

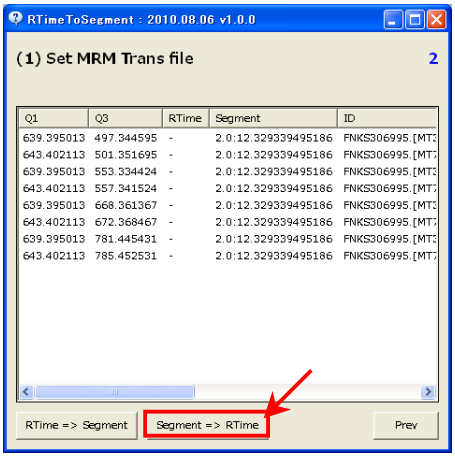
(2) When following Security Alert dialogue is popped up, click **[execute]** button.



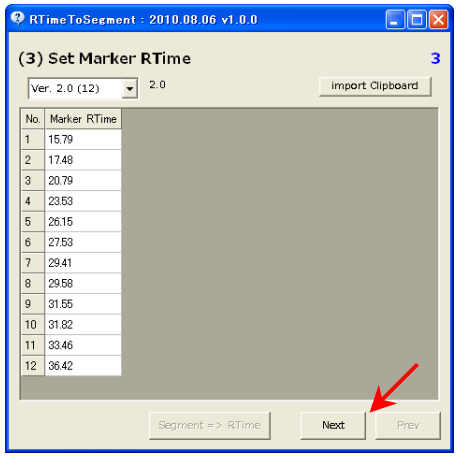
(3) Drag-and-drop method file (csv format) to the main window. Method files are included with our standard peptides.



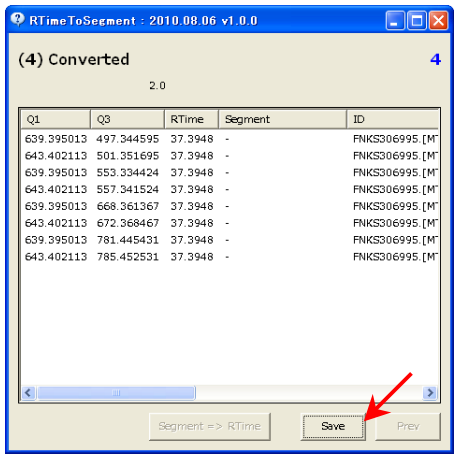
(4) Press **[Segment => Rtime]** button.



(5) Input the detected retention time of 12 peptides and press **[Next]** button.



(6) Segment values are converted into expected retention time.



(7) Press **[Save]** button and save the method file. This file is compatible with scheduled MRM mode of Analyst Software from AB SCIEX.

Windows XP(SP3), Windows 7, Windows 8 are compatible.

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