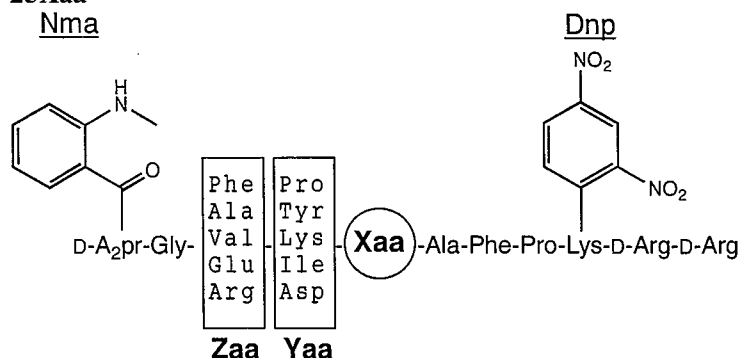


FRETS-25Xaa Series

* FRETS = Fluorescence Resonance Energy Transfer Substrates

Design of FRETS-25Xaa



Each substrate (#3701-v - #3719-v) in the FRETS-25Xaa series contains a highly fluorescent 2-(N-methylamino)benzoyl (Nma) group linked to the side chain of the amino-terminal D-A₂pr residue, which is efficiently quenched by a 2,4-dinitrophenyl (Dnp) group linked to the ε-amino function of Lys. Xaa represents a fixed position of each of the 19 natural amino acids excluding Cys (*noted in product name #3701-v - #3719-v*). A mixture of 5 amino acid residues (P, Y, K, I, and D) is at the Yaa position along with a mixture of 5 amino acid residues (F, A, V, E, and R) at the Zaa position for each fixed Xaa. This provides a peptide mixture of 25 combinations of each Xaa series resulting in a combinatorial library totaling 475 peptide substrates. Both Nma and Dnp groups are linked to the side chain of the individual residues, allowing for the determination of the cleavage site by a specific enzyme through mass spectrometric analysis and Edman degradation as well.

Principle

When an enzyme of interest cleaves any peptide bond between D-A₂pr(Nma) and Lys(Dnp) in the substrate, the fluorescence at $\lambda_{ex} = 340$ nm and $\lambda_{em} = 440$ nm increases in proportion to the release of the Nma fluorophore from the internal Dnp quencher.

Reagents

- 1) Each substrate stock solutions: each FRETS-25Xaa (#3701-v - #3719-v) in 1.0 ml of DMSO (1 mM, total of peptides)
- 2) Reference compounds stock solution: a 1:1 mixture of two solutions of #3720-v and #3721-v, each of which is reconstituted by dissolving peptides in 0.5 ml of DMSO at the concentration of 2 mM (1 mM, each reference compound)
- 3) Enzyme solution: an enzyme of interest in an appropriate buffer
- 4) Buffer

Procedure for the deduction of the substrate specificity of an enzyme with unidentified cleavage specificity

Choose the proper conditions for the measurement, such as substrate concentration and sensitivity setting, depending on the purpose of the experiment and the instrument available. Described here is one of the recommended procedures for determining the enzymatic cleavage site by the combination of the fluorometric analysis and liquid chromatography-mass spectrometry (LC-MS) analysis.

i) Primary screening: selection of the favored Xaa

·Substrate solution for primary screening (PS solution): Dilute 20 μ l of each of the above substrate stock solution with 1980 μ l of an appropriate buffer (10 μ M)

·Reference compounds solution for primary screening (PR solution): Dilute 20 μ l of the above reference compounds stock solution with 1980 μ l of an appropriate buffer (10 μ M)

- 1) Set a fluorescence spectrophotometer at $\lambda_{ex} = 340$ nm and $\lambda_{em} = 440$ nm
- 2) Mix one of the PS solution and the PR solution in ratios of 10/0, 9/1, 8/2, 5/5 and 0/10

- 3) Measure the fluorescence of the prepared solutions to obtain the calibration curve for the cleaved products
- 4) Pipette 200 μl each of all PS solutions into the cells and incubate them in the fluorescence spectrophotometer for 3 min (temperature equilibration)
- 5) Measure the fluorescence of each solution (initial fluorescence blank)
- 6) Add an appropriate volume of enzyme solution
- 7) Record the increase of the fluorescence intensity
- 8) Terminate the enzymatic reaction by using a proper inhibitor (leupeptin, E-64, pepstatin, EDTA and so on) or changing the pH of the reaction medium (using TCA, AcOH, NaOH and so on)
- 9) Choose the best Xaa-containing substrate for secondary screening

ii) Secondary screening: identification of the specificity of the enzyme (I)

·Substrate solution for secondary screening (SS solution): Dilute 200 μl of the stock solution of the best Xaa-containing substrate chosen by the above primary screening with 1800 μl of an appropriate buffer (100 μM)

·Reference compounds solution for secondary screening (SR solution): Dilute 200 μl of the above reference compounds stock solution with 1800 μl of an appropriate buffer (100 μM)

- 1) Set a fluorescence spectrophotometer at $\lambda_{\text{ex}} = 340 \text{ nm}$ and $\lambda_{\text{em}} = 440 \text{ nm}$
- 2) Mix the SS solution and the SR solution in ratios of 100/0, 95/5, 90/10, 80/20, 50/50 and 0/100
- 3) Measure the fluorescence of the prepared solutions to obtain the calibration curve for the cleaved products
- 4) Pipette 200 μl of the SS solution into the cells and incubate them in the fluorescence spectrophotometer for 3 min (temperature equilibration)
- 5) Measure the fluorescence of each solution (initial fluorescence blank)
- 6) Add an appropriate volume of enzyme solution
- 7) Record the increase of the fluorescence intensity
- 8) Terminate the enzymatic reaction by using a proper inhibitor or changing the pH of the reaction medium upon completion of the reaction at the points of 0%, 5%, 10% and 20% of the total
- 9) Subject 100 μl aliquots to LC-MS

iii) LC-MS: identification of the specificity of the enzyme (II)

·Analytical conditions

column: ODS

eluant: A) H_2O containing 0.05% TFA, B) CH_3CN containing 0.05% TFA

gradient: 10% to 40% B) in A) over 50 min

detection: UV at 220 nm and 400 nm or fluorescence

- 1) Inject 100 μl aliquots of each terminated solution at different stage of the reaction
- 2) Measure the MW of the cleaved product(s) in the peak(s) with the absorbance at 220 nm but not with 400 nm [identification of the N-terminal segment(s)]
- 3) Deduce their structure from the attached list of the theoretical MW for the cleaved products

* Comment 1: If the N-terminal segment has the identical retention time to the C-terminal segment or one of the starting uncleaved substrates, detection of the products by fluorescence is recommended.

* Comment 2: In the accidental case where the two products with the same MW (ex. Zaa-Yaa=Phe-Asp and Val-Tyr, Glu-Asp and Phe-Pro) are generated from one of the substrate, their analyses should be carried out by MS-MS sequencing and/or by Edman degradation.

Usefulness and limitation of FRET-25Xaa series for screening of substrate specificities of proteases

We have confirmed that FRET-25Xaa series are effectively used for the assay of numerous proteases such as trypsin, chymotrypsin, elastase, thrombin, papain, calpain, pepsin and thermolysin. However, they did not work well for the assay of caspase-3 and furin, probably because they have only three changeable sites (Zaa-Yaa-Xaa) in each substrate (deficiency of P4 site). This fact implies that FRET-25Xaa might not be applicable to the assay of an enzyme with wide range interacting sites with substrate.

FRETS-25Ile	Average	Monoisotopic	FRETS-25Ile	Average	Monoisotopic	FRETS-25Ile	Average	Monoisotopic	FRETS-25Ile	Average	Monoisotopic
A2pr (Nma) G	294.31	294.1328	A2pr (Nma) GAKI	606.71	606.3489	PK (Dnp) rr	721.77	721.3620	A2pr (Nma) GVIYAF	888.02	887.4541
A2pr (Nma) GA	365.38	365.1699	A2pr (Nma) GRY	613.67	613.2972	A2pr (Nma) GEDI A	722.74	722.3235	A2pr (Nma) GAP IAFP	891.02	890.4650
A2pr (Nma) GV	393.44	393.2012	A2pr (Nma) GVII	619.75	619.3693	A2pr (Nma) GFPI A	722.83	722.3752	A2pr (Nma) GRI IAF	895.06	894.5076
A2pr (Nma) GE	423.42	423.1754	A2pr (Nma) GVDI	621.68	621.3122	A2pr (Nma) GRYI	726.82	726.3813	A2pr (Nma) GRDI AF	896.99	896.4505
A2pr (Nma) GF	441.48	441.2012	K (Dnp) rr	624.65	624.3092	A2pr (Nma) GRPI A	731.84	731.4079	A2pr (Nma) GFK IAF	901.06	900.4858
A2pr (Nma) GR	450.49	450.2339	A2pr (Nma) GEPI	633.69	633.3122	A2pr (Nma) GEK I A	735.83	735.3915	A2pr (Nma) GAI IAFP	907.07	906.4963
A2pr (Nma) GAP	462.50	462.2227	A2pr (Nma) GVKI	634.77	634.3802	A2pr (Nma) GFII A	738.87	738.4065	A2pr (Nma) GADI AAFP	909.00	908.4392
A2pr (Nma) GAI	478.54	478.2540	A2pr (Nma) GAYI	641.72	641.3173	A2pr (Nma) GFDI A	740.80	740.3493	A2pr (Nma) GRK IAF	910.07	909.5185
A2pr (Nma) GAD	480.47	480.1969	A2pr (Nma) GAP I A	646.74	646.3439	A2pr (Nma) GVIY I A	740.85	740.3857	A2pr (Nma) GEY IAF	918.00	917.4283
A2pr (Nma) GVP	490.55	490.2540	A2pr (Nma) GEII	649.74	649.3435	A2pr (Nma) GRI I A	747.89	747.4392	A2pr (Nma) GVP IAFP	919.08	918.4963
A2pr (Nma) GAK	493.56	493.2649	A2pr (Nma) GEDI	651.67	651.2864	A2pr (Nma) GRDI A	749.82	749.3820	A2pr (Nma) GAK IAFP	922.08	921.5072
A2pr (Nma) GVI	506.60	506.2853	A2pr (Nma) GFP I	651.75	651.3380	A2pr (Nma) GFK I A	753.89	753.4174	A2pr (Nma) GVI IAFP	935.12	934.5276
A2pr (Nma) GVD	508.52	508.2282	A2pr (Nma) GRPI	660.76	660.3707	A2pr (Nma) GRK I A	762.90	762.4501	A2pr (Nma) GFY IAF	936.06	935.4541
A2pr (Nma) GEP	520.54	520.2282	A2pr (Nma) GAI I A	662.78	662.3752	A2pr (Nma) GEY I A	770.83	770.3599	A2pr (Nma) GVD IAFP	937.05	936.4705
A2pr (Nma) GVK	521.61	521.2962	A2pr (Nma) GADI A	664.71	664.3180	A2pr (Nma) GFY I A	788.89	788.3857	AFPK (Dnp) rr	940.02	939.4675
A2pr (Nma) GAY	528.56	528.2332	A2pr (Nma) GEK I	664.75	664.3544	A2pr (Nma) GAP IAF	793.91	793.4123	A2pr (Nma) GRY IAF	945.07	944.4868
A2pr (Nma) GEI	536.58	536.2595	Ac-K (Dnp) rr	666.69	666.3198	A2pr (Nma) GRY I A	797.90	797.4184	A2pr (Nma) GEPI AAFP	949.06	948.4705
A2pr (Nma) GED	538.51	538.2023	A2pr (Nma) GFII	667.80	667.3693	A2pr (Nma) GAI IAF	809.95	809.4436	A2pr (Nma) GVK IAFP	950.13	949.5385
A2pr (Nma) GFP	538.60	538.2540	A2pr (Nma) GFDI	669.73	669.3122	A2pr (Nma) GADI AAFP	811.88	811.3865	A2pr (Nma) GAY IAFP	957.08	956.4756
A2pr (Nma) GRP	547.61	547.2867	A2pr (Nma) GVIY I	669.77	669.3486	A2pr (Nma) GVP IAF	821.96	821.4436	A2pr (Nma) GEI IAFP	965.10	964.5018
A2pr (Nma) GEK	551.59	551.2704	A2pr (Nma) GVP I A	674.79	674.3752	A2pr (Nma) GAK IAF	824.97	824.4545	A2pr (Nma) GEDI AAFP	967.03	966.4447
A2pr (Nma) GFI	554.64	554.2853	A2pr (Nma) GRI I	676.81	676.4020	A2pr (Nma) GVI IAF	838.00	837.4749	A2pr (Nma) GFPI AAFP	967.12	966.4963
A2pr (Nma) GFD	556.57	556.2282	A2pr (Nma) GAK I A	677.79	677.3861	A2pr (Nma) GVD IAF	839.93	839.4178	A2pr (Nma) GRPI AAFP	976.13	975.5290
A2pr (Nma) GVIY	556.61	556.2645	A2pr (Nma) GRDI	678.74	678.3449	A2pr (Nma) GEPI AAFP	851.94	851.4178	A2pr (Nma) GEK IAFP	980.12	979.5127
A2pr (Nma) GRI	563.65	563.3180	A2pr (Nma) GFK I	682.81	682.3802	A2pr (Nma) GVK IAF	853.02	852.4858	A2pr (Nma) GFII AAFP	983.16	982.5276
A2pr (Nma) GRD	565.58	565.2609	A2pr (Nma) GVI I A	690.83	690.4065	A2pr (Nma) GAY IAF	859.97	859.4228	A2pr (Nma) GFD IAFP	985.09	984.4705
A2pr (Nma) GFK	569.65	569.2962	A2pr (Nma) GRK I	691.82	691.4129	A2pr (Nma) GEI IAF	867.99	867.4491	A2pr (Nma) GVIY IAFP	985.14	984.5069
A2pr (Nma) GAP I	575.66	575.3067	A2pr (Nma) GVD I A	692.76	692.3493	FPK (Dnp) rr	868.94	868.4304	A2pr (Nma) GRI IAFP	992.17	991.5603
A2pr (Nma) GRK	578.66	578.3289	A2pr (Nma) GEY I	699.75	699.3228	A2pr (Nma) GEDI AAFP	869.92	869.3919	A2pr (Nma) GRDI AAFP	994.10	993.5032
A2pr (Nma) GEY	586.59	586.2387	A2pr (Nma) GEPI A	704.77	704.3493	A2pr (Nma) GFPI AAFP	870.00	869.4436	A2pr (Nma) GFK IAFP	998.18	997.5385
A2pr (Nma) GAI I	591.70	591.3380	A2pr (Nma) GVK I A	705.85	705.4174	A2pr (Nma) GRPI AAFP	879.02	878.4763	A2pr (Nma) GRK IAFP	1007.19	1006.5712
A2pr (Nma) GADI	593.63	593.2809	A2pr (Nma) GAY I A	712.79	712.3544	A2pr (Nma) GEK IAF	883.00	882.4600	A2pr (Nma) GEY IAFP	1015.12	1014.4811
A2pr (Nma) GVP I	603.71	603.3380	A2pr (Nma) GFY I	717.81	717.3486	A2pr (Nma) GFII AAFP	886.05	885.4749	A2pr (Nma) GFY IAFP	1033.18	1032.5069
A2pr (Nma) GFY	604.65	604.2645	A2pr (Nma) GEI I A	720.81	720.3806	A2pr (Nma) GFD IAF	887.98	887.4178	A2pr (Nma) GRY IAF	1042.19	1041.5396

FRETs-25Ile	Average	Monoisotopic	FRETs-25Ile	Average	Monoisotopic	FRETs-25Ile	Average	Monoisotopic	FRETs-25Ile	Average	Monoisotopic
IAFPK (Dnp) rr	1053. 17	1052. 5516	A2pr (Nma) GR1IAFPK (Dnp)	1286. 44	1285. 6568	A2pr (Nma) GA1IAFPK (Dnp) r	1357. 52	1356. 6939	A2pr (Nma) GRKIAFPK (Dnp) r	1457. 64	1456. 7688
PIAFPK (Dnp) rr	1150. 29	1149. 6043	AYIAFPK (Dnp) rr	1287. 43	1286. 6520	A2pr (Nma) GADIAFPK (Dnp) r	1359. 45	1358. 6367	A2pr (Nma) GEYIAFPK (Dnp) r	1465. 57	1464. 6786
IIAFPK (Dnp) rr	1166. 33	1165. 6356	A2pr (Nma) GRDIAFPK (Dnp)	1288. 37	1287. 5996	FYIAFPK (Dnp) rr	1363. 52	1362. 6833	A2pr (Nma) GFYIAFPK (Dnp) r	1483. 63	1482. 7044
DIAFPK (Dnp) rr	1168. 26	1167. 5785	A2pr (Nma) GFKIAFPK (Dnp)	1292. 44	1291. 6350	GRPIAFPK (Dnp) rr	1363. 53	1362. 7269	A2pr (Nma) GRYIAFPK (Dnp) r	1492. 64	1491. 7371
KIAFPK (Dnp) rr	1181. 35	1180. 6465	GA1IAFPK (Dnp) rr	1294. 46	1293. 6942	GEKIAFPK (Dnp) rr	1367. 51	1366. 7106	A2pr (Nma) GAPIAFPK (Dnp) rr	1497. 66	1496. 7637
A2pr (Nma) GAPIAFPK (Dnp)	1185. 29	1184. 5615	E1IAFPK (Dnp) rr	1295. 45	1294. 6782	A2pr (Nma) GVP1AFPK (Dnp) r	1369. 53	1368. 6939	A2pr (Nma) GA1IAFPK (Dnp) rr	1513. 70	1512. 7950
A2pr (Nma) GA1IAFPK (Dnp)	1201. 33	1200. 5928	GADIAFPK (Dnp) rr	1296. 39	1295. 6371	GF1IAFPK (Dnp) rr	1370. 56	1369. 7255	A2pr (Nma) GADIAFPK (Dnp) rr	1515. 63	1514. 7379
A2pr (Nma) GADIAFPK (Dnp)	1203. 26	1202. 5356	EDIAFPK (Dnp) rr	1297. 38	1296. 6211	GF DIAFPK (Dnp) rr	1372. 49	1371. 6684	A2pr (Nma) GVP1AFPK (Dnp) rr	1525. 71	1524. 7950
A2pr (Nma) GVP1AFPK (Dnp)	1213. 34	1212. 5928	FPIAFPK (Dnp) rr	1297. 46	1296. 6727	A2pr (Nma) GAKIAFPK (Dnp) r	1372. 53	1371. 7048	A2pr (Nma) GAKIAFPK (Dnp) rr	1528. 72	1527. 8059
A2pr (Nma) GAKIAFPK (Dnp)	1216. 34	1215. 6037	A2pr (Nma) GRKIAFPK (Dnp)	1301. 45	1300. 6677	GVY1AFPK (Dnp) rr	1372. 53	1371. 7048	A2pr (Nma) GV1IAFPK (Dnp) rr	1541. 75	1540. 8263
YIAFPK (Dnp) rr	1216. 35	1215. 6149	GVP1AFPK (Dnp) rr	1306. 47	1305. 6942	RYIAFPK (Dnp) rr	1372. 53	1371. 7160	A2pr (Nma) GVDIAFPK (Dnp) rr	1543. 68	1542. 7692
APIAFPK (Dnp) rr	1221. 37	1220. 6414	RP1AFPK (Dnp) rr	1306. 48	1305. 7054	GR1IAFPK (Dnp) rr	1379. 57	1378. 7582	A2pr (Nma) GEPIAFPK (Dnp) rr	1555. 69	1554. 7692
A2pr (Nma) GV1IAFPK (Dnp)	1229. 38	1228. 6241	A2pr (Nma) GEYIAFPK (Dnp)	1309. 38	1308. 5775	GRDIAFPK (Dnp) rr	1381. 50	1380. 7011	A2pr (Nma) GVKIAFPK (Dnp) rr	1556. 77	1555. 8372
A2pr (Nma) GVDIAFPK (Dnp)	1231. 31	1230. 5669	GAKIAFPK (Dnp) rr	1309. 48	1308. 7051	A2pr (Nma) GV1IAFPK (Dnp) r	1385. 57	1384. 7252	A2pr (Nma) GAYIAFPK (Dnp) rr	1563. 72	1562. 7742
AI1AFPK (Dnp) rr	1237. 41	1236. 6727	EK1AFPK (Dnp) rr	1310. 46	1309. 6891	GFKIAFPK (Dnp) rr	1385. 57	1384. 7364	A2pr (Nma) GE1IAFPK (Dnp) rr	1571. 74	1570. 8005
AD1AFPK (Dnp) rr	1239. 34	1238. 6156	F1IAFPK (Dnp) rr	1313. 51	1312. 7040	A2pr (Nma) GVDIAFPK (Dnp) r	1387. 50	1386. 6680	A2pr (Nma) GEDIAFPK (Dnp) rr	1573. 67	1572. 7433
A2pr (Nma) GEPIAFPK (Dnp)	1243. 32	1242. 5669	FDIAFPK (Dnp) rr	1315. 44	1314. 6469	GRKIAFPK (Dnp) rr	1394. 58	1393. 7691	A2pr (Nma) GFPIAFPK (Dnp) rr	1573. 75	1572. 7950
A2pr (Nma) GVKIAFPK (Dnp)	1244. 40	1243. 6350	YYIAFPK (Dnp) rr	1315. 48	1314. 6833	A2pr (Nma) GEPIAFPK (Dnp) r	1399. 51	1398. 6680	A2pr (Nma) GRPIAFPK (Dnp) rr	1582. 77	1581. 8277
VPIAFPK (Dnp) rr	1249. 42	1248. 6727	GV1IAFPK (Dnp) rr	1322. 51	1321. 7255	A2pr (Nma) GVKIAFPK (Dnp) r	1400. 58	1399. 7361	A2pr (Nma) GEKIAFPK (Dnp) rr	1586. 75	1585. 8114
A2pr (Nma) GAYIAFPK (Dnp)	1251. 35	1250. 5720	RI1AFPK (Dnp) rr	1322. 52	1321. 7367	GEYIAFPK (Dnp) rr	1402. 51	1401. 6789	A2pr (Nma) GF1IAFPK (Dnp) rr	1589. 80	1588. 8263
AK1AFPK (Dnp) rr	1252. 42	1251. 6836	GVDIAFPK (Dnp) rr	1324. 44	1323. 6684	A2pr (Nma) GAYIAFPK (Dnp) r	1407. 53	1406. 6731	A2pr (Nma) GFDIAFPK (Dnp) rr	1591. 73	1590. 7692
A2pr (Nma) GE1IAFPK (Dnp)	1259. 37	1258. 5982	RDIAFPK (Dnp) rr	1324. 45	1323. 6796	A2pr (Nma) GE1IAFPK (Dnp) r	1415. 55	1414. 6993	A2pr (Nma) GY1IAFPK (Dnp) rr	1591. 77	1590. 8055
A2pr (Nma) GEDIAFPK (Dnp)	1261. 30	1260. 5411	A2pr (Nma) GFYIAFPK (Dnp)	1327. 44	1326. 6033	A2pr (Nma) GEDIAFPK (Dnp) r	1417. 48	1416. 6422	A2pr (Nma) GR1IAFPK (Dnp) rr	1598. 81	1597. 8590
A2pr (Nma) GFPIAFPK (Dnp)	1261. 38	1260. 5928	FKIAFPK (Dnp) rr	1328. 52	1327. 7149	A2pr (Nma) GFPIAFPK (Dnp) r	1417. 57	1416. 6939	A2pr (Nma) GRDIAFPK (Dnp) rr	1600. 74	1599. 8019
VI1AFPK (Dnp) rr	1265. 46	1264. 7040	A2pr (Nma) GRYIAFPK (Dnp)	1336. 45	1335. 6360	GFY1AFPK (Dnp) rr	1420. 57	1419. 7048	A2pr (Nma) GFKIAFPK (Dnp) rr	1604. 81	1603. 8372
VDIAFPK (Dnp) rr	1267. 39	1266. 6469	GEPIAFPK (Dnp) rr	1336. 46	1335. 6684	A2pr (Nma) GRPIAFPK (Dnp) r	1426. 58	1425. 7266	A2pr (Nma) GRKIAFPK (Dnp) rr	1613. 82	1612. 8699
A2pr (Nma) GRPIAFPK (Dnp)	1270. 40	1269. 6255	GVKIAFPK (Dnp) rr	1337. 53	1336. 7364	GRYIAFPK (Dnp) rr	1429. 58	1428. 7375	A2pr (Nma) GEYIAFPK (Dnp) rr	1621. 75	1620. 7797
A2pr (Nma) GEKIAFPK (Dnp)	1274. 38	1273. 6091	RKIAFPK (Dnp) rr	1337. 53	1336. 7476	A2pr (Nma) GEKIAFPK (Dnp) r	1430. 57	1429. 7102	A2pr (Nma) GFYIAFPK (Dnp) rr	1639. 81	1638. 8055
A2pr (Nma) GF1IAFPK (Dnp)	1277. 43	1276. 6241	A2pr (Nma) GAPIAFPK (Dnp) r	1341. 47	1340. 6626	A2pr (Nma) GF1IAFPK (Dnp) r	1433. 61	1432. 7252	A2pr (Nma) GRYIAFPK (Dnp) rr	1648. 82	1647. 8382
GAPIAFPK (Dnp) rr	1278. 42	1277. 6629	GAYIAFPK (Dnp) rr	1344. 48	1343. 6735	A2pr (Nma) GFDIAFPK (Dnp) r	1435. 54	1434. 6680			
A2pr (Nma) GFDIAFPK (Dnp)	1279. 36	1278. 5669	EYIAFPK (Dnp) rr	1345. 46	1344. 6575	A2pr (Nma) GY1IAFPK (Dnp) r	1435. 58	1434. 7044			
A2pr (Nma) GY1IAFPK (Dnp)	1279. 40	1278. 6033	GE1IAFPK (Dnp) rr	1352. 50	1351. 6997	A2pr (Nma) GR1IAFPK (Dnp) r	1442. 62	1441. 7579			
EPIAFPK (Dnp) rr	1279. 40	1278. 6469	GEDIAFPK (Dnp) rr	1354. 43	1353. 6426	A2pr (Nma) GRDIAFPK (Dnp) r	1444. 55	1443. 7007			
YK1AFPK (Dnp) rr	1280. 48	1279. 7149	GFPIAFPK (Dnp) rr	1354. 51	1353. 6942	A2pr (Nma) GFKIAFPK (Dnp) r	1448. 63	1447. 7361			