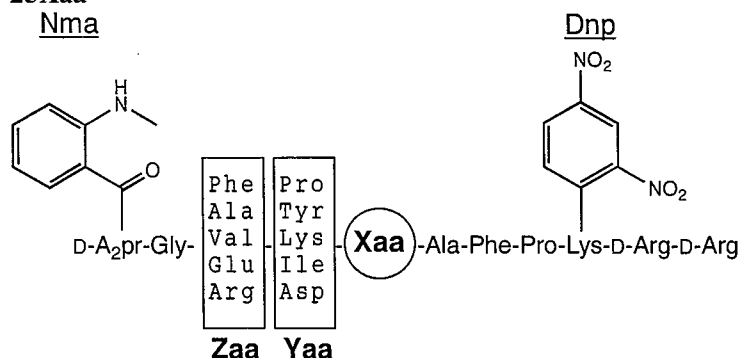


## FRETS-25Xaa Series

\* 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  $\epsilon$ -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  $\mu$ l of each of the above substrate stock solution with 1980  $\mu$ l of an appropriate buffer (10  $\mu$ M)

·Reference compounds solution for primary screening (PR solution): Dilute 20  $\mu$ l of the above reference compounds stock solution with 1980  $\mu$ l of an appropriate buffer (10  $\mu$ 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  $\mu\text{l}$  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  $\mu\text{l}$  of the stock solution of the best Xaa-containing substrate chosen by the above primary screening with 1800  $\mu\text{l}$  of an appropriate buffer (100  $\mu\text{M}$ )

·Reference compounds solution for secondary screening (SR solution): Dilute 200  $\mu\text{l}$  of the above reference compounds stock solution with 1800  $\mu\text{l}$  of an appropriate buffer (100  $\mu\text{M}$ )

- 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  $\mu\text{l}$  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  $\mu\text{l}$  aliquots to LC-MS

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

·Analytical conditions

column: ODS

eluant: A)  $\text{H}_2\text{O}$  containing 0.05% TFA, B)  $\text{CH}_3\text{CN}$  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  $\mu\text{l}$  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-25Leu	Average	Monoisotopic	FRETS-25Leu	Average	Monoisotopic	FRETS-25Leu	Average	Monoisotopic	FRETS-25Leu	Average	Monoisotopic
A2pr (Nma) G	294.31	294.1328	A2pr (Nma) GAKL	606.71	606.3489	PK (Dnp) rr	721.77	721.3620	A2pr (Nma) GVYLAF	888.02	887.4541
A2pr (Nma) GA	365.38	365.1699	A2pr (Nma) GRY	613.67	613.2972	A2pr (Nma) GEDLA	722.74	722.3235	A2pr (Nma) GAPLAF	891.02	890.4650
A2pr (Nma) GV	393.44	393.2012	A2pr (Nma) GVIL	619.75	619.3693	A2pr (Nma) GFPLA	722.83	722.3752	A2pr (Nma) GRILAF	895.06	894.5076
A2pr (Nma) GE	423.42	423.1754	A2pr (Nma) GVDL	621.68	621.3122	A2pr (Nma) GRYL	726.82	726.3813	A2pr (Nma) GRDLAF	896.99	896.4505
A2pr (Nma) GF	441.48	441.2012	K (Dnp) rr	624.65	624.3092	A2pr (Nma) GRPLA	731.84	731.4079	A2pr (Nma) GFKLAF	901.06	900.4858
A2pr (Nma) GR	450.49	450.2339	A2pr (Nma) GEPL	633.69	633.3122	A2pr (Nma) GEKLA	735.83	735.3915	A2pr (Nma) GAILAF	907.07	906.4963
A2pr (Nma) GAP	462.50	462.2227	A2pr (Nma) GVKL	634.77	634.3802	A2pr (Nma) GFILA	738.87	738.4065	A2pr (Nma) GADLAF	909.00	908.4392
A2pr (Nma) GAI	478.54	478.2540	A2pr (Nma) GAYL	641.72	641.3173	A2pr (Nma) GFDLA	740.80	740.3493	A2pr (Nma) GRKLAF	910.07	909.5185
A2pr (Nma) GAD	480.47	480.1969	A2pr (Nma) GAPLA	646.74	646.3439	A2pr (Nma) GVYLA	740.85	740.3857	A2pr (Nma) GEYLAF	918.00	917.4283
A2pr (Nma) GVP	490.55	490.2540	A2pr (Nma) GEIL	649.74	649.3435	A2pr (Nma) GRILA	747.89	747.4392	A2pr (Nma) GVPLAF	919.08	918.4963
A2pr (Nma) GAK	493.56	493.2649	A2pr (Nma) GEDL	651.67	651.2864	A2pr (Nma) GRDLA	749.82	749.3820	A2pr (Nma) GAKLAF	922.08	921.5072
A2pr (Nma) GVI	506.60	506.2853	A2pr (Nma) GFPL	651.75	651.3380	A2pr (Nma) GFKLA	753.89	753.4174	A2pr (Nma) GVILAF	935.12	934.5276
A2pr (Nma) GVD	508.52	508.2282	A2pr (Nma) GRPL	660.76	660.3707	A2pr (Nma) GRKLA	762.90	762.4501	A2pr (Nma) GFYLAF	936.06	935.4541
A2pr (Nma) GEP	520.54	520.2282	A2pr (Nma) GAILA	662.78	662.3752	A2pr (Nma) GEYLA	770.83	770.3599	A2pr (Nma) GVDLAF	937.05	936.4705
A2pr (Nma) GVK	521.61	521.2962	A2pr (Nma) GADLA	664.71	664.3180	A2pr (Nma) GFYLA	788.89	788.3857	AFPK (Dnp) rr	940.02	939.4675
A2pr (Nma) GAY	528.56	528.2332	A2pr (Nma) GEKL	664.75	664.3544	A2pr (Nma) GAPLAF	793.91	793.4123	A2pr (Nma) GRYLAF	945.07	944.4868
A2pr (Nma) GEI	536.58	536.2595	Ac-K (Dnp) rr	666.69	666.3198	A2pr (Nma) GRYLA	797.90	797.4184	A2pr (Nma) GEPLAF	949.06	948.4705
A2pr (Nma) GED	538.51	538.2023	A2pr (Nma) GFIL	667.80	667.3693	A2pr (Nma) GAILAF	809.95	809.4436	A2pr (Nma) GVKLAF	950.13	949.5385
A2pr (Nma) GFP	538.60	538.2540	A2pr (Nma) GFDL	669.73	669.3122	A2pr (Nma) GADLAF	811.88	811.3865	A2pr (Nma) GAYLAF	957.08	956.4756
A2pr (Nma) GRP	547.61	547.2867	A2pr (Nma) GVYL	669.77	669.3486	A2pr (Nma) GVPLAF	821.96	821.4436	A2pr (Nma) GEILAF	965.10	964.5018
A2pr (Nma) GEK	551.59	551.2704	A2pr (Nma) GVPLA	674.79	674.3752	A2pr (Nma) GAKLAF	824.97	824.4545	A2pr (Nma) GEDLAF	967.03	966.4447
A2pr (Nma) GFI	554.64	554.2853	A2pr (Nma) GRIL	676.81	676.4020	A2pr (Nma) GVILAF	838.00	837.4749	A2pr (Nma) GFPLAF	967.12	966.4963
A2pr (Nma) GFD	556.57	556.2282	A2pr (Nma) GAKLA	677.79	677.3861	A2pr (Nma) GVDLAF	839.93	839.4178	A2pr (Nma) GRPLAF	976.13	975.5290
A2pr (Nma) GVY	556.61	556.2645	A2pr (Nma) GRDL	678.74	678.3449	A2pr (Nma) GEPLAF	851.94	851.4178	A2pr (Nma) GEKLAF	980.12	979.5127
A2pr (Nma) GRI	563.65	563.3180	A2pr (Nma) GFKL	682.81	682.3802	A2pr (Nma) GVKLAF	853.02	852.4858	A2pr (Nma) GFILAF	983.16	982.5276
A2pr (Nma) GRD	565.58	565.2609	A2pr (Nma) GVILA	690.83	690.4065	A2pr (Nma) GAYLAF	859.97	859.4228	A2pr (Nma) GFDLAF	985.09	984.4705
A2pr (Nma) GFK	569.65	569.2962	A2pr (Nma) GRKL	691.82	691.4129	A2pr (Nma) GEILAF	867.99	867.4491	A2pr (Nma) GVYLAF	985.14	984.5069
A2pr (Nma) GAPL	575.66	575.3067	A2pr (Nma) GVDLA	692.76	692.3493	FPK (Dnp) rr	868.94	868.4304	A2pr (Nma) GRILAF	992.17	991.5603
A2pr (Nma) GRK	578.66	578.3289	A2pr (Nma) GEYL	699.75	699.3228	A2pr (Nma) GEDLAF	869.92	869.3919	A2pr (Nma) GRDLAF	994.10	993.5032
A2pr (Nma) GEY	586.59	586.2387	A2pr (Nma) GEPLA	704.77	704.3493	A2pr (Nma) GFPLAF	870.00	869.4436	A2pr (Nma) GFKLAF	998.18	997.5385
A2pr (Nma) GAIL	591.70	591.3380	A2pr (Nma) GVKLA	705.85	705.4174	A2pr (Nma) GRPLAF	879.02	878.4763	A2pr (Nma) GRKLAF	1007.19	1006.5712
A2pr (Nma) GADL	593.63	593.2809	A2pr (Nma) GAYLA	712.79	712.3544	A2pr (Nma) GEKLAF	883.00	882.4600	A2pr (Nma) GEYLAF	1015.12	1014.4811
A2pr (Nma) GVPL	603.71	603.3380	A2pr (Nma) GFYL	717.81	717.3486	A2pr (Nma) GFILAF	886.05	885.4749	A2pr (Nma) GFYLAF	1033.18	1032.5069
A2pr (Nma) GFY	604.65	604.2645	A2pr (Nma) GEILA	720.81	720.3806	A2pr (Nma) GFDLAF	887.98	887.4178	A2pr (Nma) GRYLAF	1042.19	1041.5396

FRETs-25Leu	Average	Monoisotopic	FRETs-25Leu	Average	Monoisotopic	FRETs-25Leu	Average	Monoisotopic	FRETs-25Leu	Average	Monoisotopic
LAFPK (Dnp) rr	1053. 17	1052. 5516	A2pr (Nma) GRILAFPK (Dnp)	1286. 44	1285. 6568	A2pr (Nma) GAILAFPK (Dnp) r	1357. 52	1356. 6939	A2pr (Nma) GRKLAFPK (Dnp) r	1457. 64	1456. 7688
PLAFPK (Dnp) rr	1150. 29	1149. 6043	AYLAFPK (Dnp) rr	1287. 43	1286. 6520	A2pr (Nma) GADLAFPK (Dnp) r	1359. 45	1358. 6367	A2pr (Nma) GEYLAFPK (Dnp) r	1465. 57	1464. 6786
ILAFPK (Dnp) rr	1166. 33	1165. 6356	A2pr (Nma) GRDLAFPK (Dnp)	1288. 37	1287. 5996	FYLAFPK (Dnp) rr	1363. 52	1362. 6833	A2pr (Nma) GFYLAFPK (Dnp) r	1483. 63	1482. 7044
DLAFPK (Dnp) rr	1168. 26	1167. 5785	A2pr (Nma) GFKLAFPK (Dnp)	1292. 44	1291. 6350	GRPLAFPK (Dnp) rr	1363. 53	1362. 7269	A2pr (Nma) GRYLAFPK (Dnp) r	1492. 64	1491. 7371
KLAFPK (Dnp) rr	1181. 35	1180. 6465	GAILAFPK (Dnp) rr	1294. 46	1293. 6942	GEKLAFPK (Dnp) rr	1367. 51	1366. 7106	A2pr (Nma) GAPLAFPK (Dnp) rr	1497. 66	1496. 7637
A2pr (Nma) GAPLAFPK (Dnp)	1185. 29	1184. 5615	EILAFPK (Dnp) rr	1295. 45	1294. 6782	A2pr (Nma) GVLAFPK (Dnp) r	1369. 53	1368. 6939	A2pr (Nma) GAILAFPK (Dnp) rr	1513. 70	1512. 7950
A2pr (Nma) GAILAFPK (Dnp)	1201. 33	1200. 5928	GADLAFPK (Dnp) rr	1296. 39	1295. 6371	GFILAFPK (Dnp) rr	1370. 56	1369. 7255	A2pr (Nma) GADLAFPK (Dnp) rr	1515. 63	1514. 7379
A2pr (Nma) GADLAFPK (Dnp)	1203. 26	1202. 5356	EDLAFPK (Dnp) rr	1297. 38	1296. 6211	GFDLAFPK (Dnp) rr	1372. 49	1371. 6684	A2pr (Nma) GVLAFPK (Dnp) rr	1525. 71	1524. 7950
A2pr (Nma) GVLAFPK (Dnp)	1213. 34	1212. 5928	FPLAFPK (Dnp) rr	1297. 46	1296. 6727	A2pr (Nma) GAKLAFPK (Dnp) r	1372. 53	1371. 7048	A2pr (Nma) GAKLAFPK (Dnp) rr	1528. 72	1527. 8059
A2pr (Nma) GAKLAFPK (Dnp)	1216. 34	1215. 6037	A2pr (Nma) GRKLAFPK (Dnp)	1301. 45	1300. 6677	GVYLAFPK (Dnp) rr	1372. 53	1371. 7048	A2pr (Nma) GVILAFPK (Dnp) rr	1541. 75	1540. 8263
YLAFPK (Dnp) rr	1216. 35	1215. 6149	GVPLAFPK (Dnp) rr	1306. 47	1305. 6942	RYLAFPK (Dnp) rr	1372. 53	1371. 7160	A2pr (Nma) GVDLAFPK (Dnp) rr	1543. 68	1542. 7692
APLAFPK (Dnp) rr	1221. 37	1220. 6414	RPLAFPK (Dnp) rr	1306. 48	1305. 7054	GRILAFPK (Dnp) rr	1379. 57	1378. 7582	A2pr (Nma) GEPLAFPK (Dnp) rr	1555. 69	1554. 7692
A2pr (Nma) GVILAFPK (Dnp)	1229. 38	1228. 6241	A2pr (Nma) GEYLAFPK (Dnp)	1309. 38	1308. 5775	GRDLAFPK (Dnp) rr	1381. 50	1380. 7011	A2pr (Nma) GVKLAFPK (Dnp) rr	1556. 77	1555. 8372
A2pr (Nma) GVDLAFPK (Dnp)	1231. 31	1230. 5669	GAKLAFPK (Dnp) rr	1309. 48	1308. 7051	A2pr (Nma) GVILAFPK (Dnp) r	1385. 57	1384. 7252	A2pr (Nma) GAYLAFPK (Dnp) rr	1563. 72	1562. 7742
A1LAFPK (Dnp) rr	1237. 41	1236. 6727	EKLAFPK (Dnp) rr	1310. 46	1309. 6891	GFKLAFPK (Dnp) rr	1385. 57	1384. 7364	A2pr (Nma) GEILAFPK (Dnp) rr	1571. 74	1570. 8005
ADLAFPK (Dnp) rr	1239. 34	1238. 6156	FILAFPK (Dnp) rr	1313. 51	1312. 7040	A2pr (Nma) GVDLAFPK (Dnp) r	1387. 50	1386. 6680	A2pr (Nma) GEDLAFPK (Dnp) rr	1573. 67	1572. 7433
A2pr (Nma) GEPLAFPK (Dnp)	1243. 32	1242. 5669	FDLAFPK (Dnp) rr	1315. 44	1314. 6469	GRKLAFPK (Dnp) rr	1394. 58	1393. 7691	A2pr (Nma) GFPLAFPK (Dnp) rr	1573. 75	1572. 7950
A2pr (Nma) GVKLAFPK (Dnp)	1244. 40	1243. 6350	VYLAFPK (Dnp) rr	1315. 48	1314. 6833	A2pr (Nma) GEPLAFPK (Dnp) r	1399. 51	1398. 6680	A2pr (Nma) GRPLAFPK (Dnp) rr	1582. 77	1581. 8277
VPLAFPK (Dnp) rr	1249. 42	1248. 6727	GVILAFPK (Dnp) rr	1322. 51	1321. 7255	A2pr (Nma) GVKLAFPK (Dnp) r	1400. 58	1399. 7361	A2pr (Nma) GEKLAFPK (Dnp) rr	1586. 75	1585. 8114
A2pr (Nma) GAYLAFPK (Dnp)	1251. 35	1250. 5720	RILAFPK (Dnp) rr	1322. 52	1321. 7367	GEYLAFPK (Dnp) rr	1402. 51	1401. 6789	A2pr (Nma) GFILAFPK (Dnp) rr	1589. 80	1588. 8263
AKLAFPK (Dnp) rr	1252. 42	1251. 6836	GVDLAFPK (Dnp) rr	1324. 44	1323. 6684	A2pr (Nma) GAYLAFPK (Dnp) r	1407. 53	1406. 6731	A2pr (Nma) GFDLAFPK (Dnp) rr	1591. 73	1590. 7692
A2pr (Nma) GEILAFPK (Dnp)	1259. 37	1258. 5982	RDLAFPK (Dnp) rr	1324. 45	1323. 6796	A2pr (Nma) GEILAFPK (Dnp) r	1415. 55	1414. 6993	A2pr (Nma) GVYLAFPK (Dnp) rr	1591. 77	1590. 8055
A2pr (Nma) GEDLAFPK (Dnp)	1261. 30	1260. 5411	A2pr (Nma) GFYLAFPK (Dnp)	1327. 44	1326. 6033	A2pr (Nma) GEDLAFPK (Dnp) r	1417. 48	1416. 6422	A2pr (Nma) GRILAFPK (Dnp) rr	1598. 81	1597. 8590
A2pr (Nma) GFPLAFPK (Dnp)	1261. 38	1260. 5928	FKLAFPK (Dnp) rr	1328. 52	1327. 7149	A2pr (Nma) GFPLAFPK (Dnp) r	1417. 57	1416. 6939	A2pr (Nma) GRDLAFPK (Dnp) rr	1600. 74	1599. 8019
VILAFPK (Dnp) rr	1265. 46	1264. 7040	A2pr (Nma) GRYLAFPK (Dnp)	1336. 45	1335. 6360	GFYLAFPK (Dnp) rr	1420. 57	1419. 7048	A2pr (Nma) GFKLAFPK (Dnp) rr	1604. 81	1603. 8372
VDLAFPK (Dnp) rr	1267. 39	1266. 6469	GEPLAFPK (Dnp) rr	1336. 46	1335. 6684	A2pr (Nma) GRPLAFPK (Dnp) r	1426. 58	1425. 7266	A2pr (Nma) GRKLAFPK (Dnp) rr	1613. 82	1612. 8699
A2pr (Nma) GRPLAFPK (Dnp)	1270. 40	1269. 6255	GVKLAFPK (Dnp) rr	1337. 53	1336. 7364	GRYLAFPK (Dnp) rr	1429. 58	1428. 7375	A2pr (Nma) GEYLAFPK (Dnp) rr	1621. 75	1620. 7797
A2pr (Nma) GEKLAFPK (Dnp)	1274. 38	1273. 6091	RKLAFPK (Dnp) rr	1337. 53	1336. 7476	A2pr (Nma) GEKLAFPK (Dnp) r	1430. 57	1429. 7102	A2pr (Nma) GFYLAFPK (Dnp) rr	1639. 81	1638. 8055
A2pr (Nma) GFILAFPK (Dnp)	1277. 43	1276. 6241	A2pr (Nma) GAPLAFPK (Dnp) r	1341. 47	1340. 6626	A2pr (Nma) GFILAFPK (Dnp) r	1433. 61	1432. 7252	A2pr (Nma) GRYLAFPK (Dnp) rr	1648. 82	1647. 8382
GAPLAFPK (Dnp) rr	1278. 42	1277. 6629	GAYLAFPK (Dnp) rr	1344. 48	1343. 6735	A2pr (Nma) GFDLAFPK (Dnp) r	1435. 54	1434. 6680			
A2pr (Nma) GFDLAFPK (Dnp)	1279. 36	1278. 5669	EYLAFPK (Dnp) rr	1345. 46	1344. 6575	A2pr (Nma) GVYLAFPK (Dnp) r	1435. 58	1434. 7044			
A2pr (Nma) GVYLAFPK (Dnp)	1279. 40	1278. 6033	GEILAFPK (Dnp) rr	1352. 50	1351. 6997	A2pr (Nma) GRILAFPK (Dnp) r	1442. 62	1441. 7579			
EPLAFPK (Dnp) rr	1279. 40	1278. 6469	GEDLAFPK (Dnp) rr	1354. 43	1353. 6426	A2pr (Nma) GRDLAFPK (Dnp) r	1444. 55	1443. 7007			
YKLAFPK (Dnp) rr	1280. 48	1279. 7149	GFPLAFPK (Dnp) rr	1354. 51	1353. 6942	A2pr (Nma) GFKLAFPK (Dnp) r	1448. 63	1447. 7361			