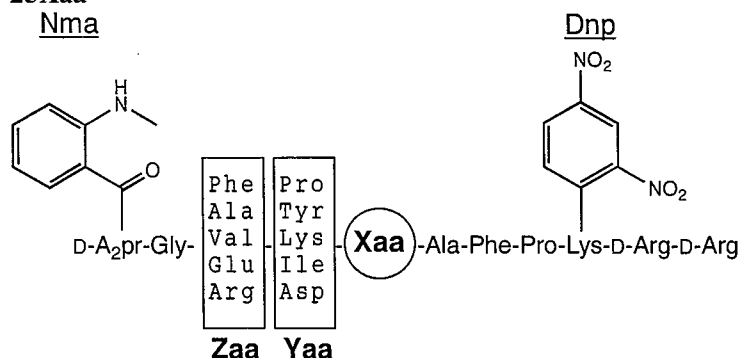


FRETS-25Xaa Series

* 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-25Tyr	Average	Monoisotopic	FRETS-25Tyr	Average	Monoisotopic	FRETS-25Tyr	Average	Monoisotopic	FRETS-25Tyr	Average	Monoisotopic
A2pr (Nma) G	294.31	294.1328	A2pr (Nma) GADY	643.64	643.2602	A2pr (Nma) GEIYA	770.83	770.3599	A2pr (Nma) GVYYAF	938.04	937.4334
A2pr (Nma) GA	365.38	365.1699	A2pr (Nma) GVPY	653.73	653.3173	A2pr (Nma) GEDYA	772.76	772.3028	AFPk (Dnp) rr	940.02	939.4675
A2pr (Nma) GV	393.44	393.2012	A2pr (Nma) GAKY	656.73	656.3282	A2pr (Nma) GFPYA	772.85	772.3544	A2pr (Nma) GAPYAFP	941.04	940.4443
A2pr (Nma) GE	423.42	423.1754	Ac-K (Dnp) rr	666.69	666.3198	A2pr (Nma) GRYYY	776.84	776.3606	A2pr (Nma) GRIYAF	945.07	944.4868
A2pr (Nma) GF	441.48	441.2012	A2pr (Nma) GV1Y	669.77	669.3486	A2pr (Nma) GRPYA	781.86	781.3871	A2pr (Nma) GRDYAF	947.00	946.4297
A2pr (Nma) GR	450.49	450.2339	A2pr (Nma) GVDY	671.70	671.2915	A2pr (Nma) GEKYA	785.84	785.3708	A2pr (Nma) GFKYAF	951.08	950.4650
A2pr (Nma) GAP	462.50	462.2227	A2pr (Nma) GEPY	683.71	683.2915	A2pr (Nma) GF1YA	788.89	788.3857	A2pr (Nma) GAIYAFP	957.08	956.4756
A2pr (Nma) GAI	478.54	478.2540	A2pr (Nma) GVKY	684.78	684.3595	A2pr (Nma) GFDYA	790.82	790.3286	A2pr (Nma) GADYAFP	959.01	958.4185
A2pr (Nma) GAD	480.47	480.1969	A2pr (Nma) GAYY	691.73	691.2966	A2pr (Nma) GVYYA	790.86	790.3650	A2pr (Nma) GRKYAF	960.09	959.4977
A2pr (Nma) GVP	490.55	490.2540	A2pr (Nma) GAPYA	696.75	696.3231	A2pr (Nma) GRIYA	797.90	797.4184	A2pr (Nma) GEYYAF	968.02	967.4076
A2pr (Nma) GAK	493.56	493.2649	A2pr (Nma) GE1Y	699.75	699.3228	A2pr (Nma) GRDYA	799.83	799.3613	A2pr (Nma) GVPYAFP	969.09	968.4756
A2pr (Nma) GVI	506.60	506.2853	A2pr (Nma) GEDY	701.68	701.2657	A2pr (Nma) GFKYA	803.90	803.3966	A2pr (Nma) GAKYAFP	972.10	971.4865
A2pr (Nma) GVD	508.52	508.2282	A2pr (Nma) GFPY	701.77	701.3173	A2pr (Nma) GRKYA	812.92	812.4293	A2pr (Nma) GV1YAFP	985.14	984.5069
A2pr (Nma) GEP	520.54	520.2282	A2pr (Nma) GRPY	710.78	710.3500	A2pr (Nma) GEYYA	820.84	820.3392	A2pr (Nma) GFYYAF	986.08	985.4334
A2pr (Nma) GVK	521.61	521.2962	A2pr (Nma) GAIYA	712.79	712.3544	A2pr (Nma) GFYYA	838.90	838.3650	A2pr (Nma) GVDYAFP	987.07	986.4498
A2pr (Nma) GAY	528.56	528.2332	A2pr (Nma) GADYA	714.72	714.2973	A2pr (Nma) GAPYAF	843.92	843.3915	A2pr (Nma) GRYYAF	995.09	994.4661
A2pr (Nma) GEI	536.58	536.2595	A2pr (Nma) GEKY	714.77	714.3337	A2pr (Nma) GRYYA	847.92	847.3977	A2pr (Nma) GEPYAFP	999.08	998.4498
A2pr (Nma) GED	538.51	538.2023	A2pr (Nma) GF1Y	717.81	717.3486	A2pr (Nma) GAIYAF	859.97	859.4228	A2pr (Nma) GVKYAFP	1000.15	999.5178
A2pr (Nma) GFP	538.60	538.2540	A2pr (Nma) GFDY	719.74	719.2915	A2pr (Nma) GADYAF	861.90	861.3657	A2pr (Nma) GAYYAFP	1007.10	1006.4549
A2pr (Nma) GRP	547.61	547.2867	A2pr (Nma) GVYY	719.78	719.3279	FPK (Dnp) rr	868.94	868.4304	A2pr (Nma) GE1YAFP	1015.12	1014.4811
A2pr (Nma) GEK	551.59	551.2704	PK (Dnp) rr	721.77	721.3620	A2pr (Nma) GVPYAF	871.98	871.4228	A2pr (Nma) GEDYAFP	1017.05	1016.4240
A2pr (Nma) GF1	554.64	554.2853	A2pr (Nma) GVPYA	724.80	724.3544	A2pr (Nma) GAKYAF	874.98	874.4337	A2pr (Nma) GFPYAFP	1017.14	1016.4756
A2pr (Nma) GFD	556.57	556.2282	A2pr (Nma) GRIY	726.82	726.3813	A2pr (Nma) GV1YAF	888.02	887.4541	A2pr (Nma) GRPYAFP	1026.15	1025.5083
A2pr (Nma) GVY	556.61	556.2645	A2pr (Nma) GAKYA	727.81	727.3653	A2pr (Nma) GVDYAF	889.95	889.3970	A2pr (Nma) GEKYAFP	1030.13	1029.4920
A2pr (Nma) GRI	563.65	563.3180	A2pr (Nma) GRDY	728.75	728.3242	A2pr (Nma) GEPYAF	901.96	901.3970	A2pr (Nma) GF1YAFP	1033.18	1032.5069
A2pr (Nma) GRD	565.58	565.2609	A2pr (Nma) GFKY	732.83	732.3595	A2pr (Nma) GVKYAF	903.03	902.4650	A2pr (Nma) GFDYAFP	1035.11	1034.4498
A2pr (Nma) GFK	569.65	569.2962	A2pr (Nma) GV1YA	740.85	740.3857	A2pr (Nma) GAYYAF	909.98	909.4021	A2pr (Nma) GVYYAFP	1035.15	1034.4862
A2pr (Nma) GRK	578.66	578.3289	A2pr (Nma) GRKY	741.84	741.3922	A2pr (Nma) GE1YAF	918.00	917.4283	A2pr (Nma) GRIYAFP	1042.19	1041.5396
A2pr (Nma) GEY	586.59	586.2387	A2pr (Nma) GVDYA	742.78	742.3286	A2pr (Nma) GEDYAF	919.93	919.3712	A2pr (Nma) GRDYAFP	1044.12	1043.4825
A2pr (Nma) GFY	604.65	604.2645	A2pr (Nma) GEYY	749.77	749.3021	A2pr (Nma) GFPYAF	920.02	919.4228	A2pr (Nma) GFKYAFP	1048.19	1047.5178
A2pr (Nma) GRY	613.67	613.2972	A2pr (Nma) GEPYA	754.79	754.3286	A2pr (Nma) GRPYAF	929.03	928.4555	A2pr (Nma) GRKYAFP	1057.20	1056.5505
K (Dnp) rr	624.65	624.3092	A2pr (Nma) GVKYA	755.86	755.3966	A2pr (Nma) GEKYAF	933.02	932.4392	A2pr (Nma) GEYYAFP	1065.13	1064.4603
A2pr (Nma) GAPY	625.67	625.2860	A2pr (Nma) GAYYA	762.81	762.3337	A2pr (Nma) GF1YAF	936.06	935.4541	A2pr (Nma) GFYYAFP	1083.19	1082.4862
A2pr (Nma) GAIY	641.72	641.3173	A2pr (Nma) GFYY	767.83	767.3279	A2pr (Nma) GFDYAF	937.99	937.3970	A2pr (Nma) GRYYAFP	1092.21	1091.5189

FRETS-25Tyr	Average	Monoisotopic	FRETS-25Tyr	Average	Monoisotopic	FRETS-25Tyr	Average	Monoisotopic	FRETS-25Tyr	Average	Monoisotopic
YAFPK (Dnp) rr	1103. 19	1102. 5308	A2pr (Nma) GR1YAFPK (Dnp)	1336. 45	1335. 6360	A2pr (Nma) GA1YAFPK (Dnp) r	1407. 53	1406. 6731	A2pr (Nma) GRKYAFPK (Dnp) r	1507. 65	1506. 7480
PYAFPK (Dnp) rr	1200. 31	1199. 5836	AYAFPK (Dnp) rr	1337. 44	1336. 6313	A2pr (Nma) GADYAFPK (Dnp) r	1409. 46	1408. 6160	A2pr (Nma) GEYAFPK (Dnp) r	1515. 58	1514. 6579
IYAFPK (Dnp) rr	1216. 35	1215. 6149	A2pr (Nma) GRDYAFPK (Dnp)	1338. 38	1337. 5789	FYYAFPK (Dnp) rr	1413. 54	1412. 6626	A2pr (Nma) GFYYAFPK (Dnp) r	1533. 64	1532. 6837
DYAFPK (Dnp) rr	1218. 28	1217. 5578	A2pr (Nma) GFKYAFPK (Dnp)	1342. 46	1341. 6142	GRPYAFPK (Dnp) rr	1413. 54	1412. 7062	A2pr (Nma) GRYYAFPK (Dnp) r	1542. 65	1541. 7164
KYAFPK (Dnp) rr	1231. 36	1230. 6258	GA1YAFPK (Dnp) rr	1344. 48	1343. 6735	GEKYAFPK (Dnp) rr	1417. 53	1416. 6898	A2pr (Nma) GAPYAFPK (Dnp) rr	1547. 67	1546. 7429
A2pr (Nma) GAPYAFPK (Dnp)	1235. 30	1234. 5407	E1YAFPK (Dnp) rr	1345. 46	1344. 6575	A2pr (Nma) GVPYAFPK (Dnp) r	1419. 54	1418. 6731	A2pr (Nma) GA1YAFPK (Dnp) rr	1563. 72	1562. 7742
A2pr (Nma) GA1YAFPK (Dnp)	1251. 35	1250. 5720	GADYAFPK (Dnp) rr	1346. 41	1345. 6163	GF1YAFPK (Dnp) rr	1420. 57	1419. 7048	A2pr (Nma) GADYAFPK (Dnp) rr	1565. 65	1564. 7171
A2pr (Nma) GADYAFPK (Dnp)	1253. 28	1252. 5149	EDYAFPK (Dnp) rr	1347. 39	1346. 6004	GFDYAFPK (Dnp) rr	1422. 50	1421. 6476	A2pr (Nma) GVPYAFPK (Dnp) rr	1575. 73	1574. 7742
A2pr (Nma) GVPYAFPK (Dnp)	1263. 36	1262. 5720	FPYAFPK (Dnp) rr	1347. 48	1346. 6520	A2pr (Nma) GAKYAFPK (Dnp) r	1422. 55	1421. 6840	A2pr (Nma) GAKYAFPK (Dnp) rr	1578. 73	1577. 7851
A2pr (Nma) GAKYAFPK (Dnp)	1266. 36	1265. 5829	A2pr (Nma) GRKYAFPK (Dnp)	1351. 47	1350. 6469	GVYYAFPK (Dnp) rr	1422. 55	1421. 6840	A2pr (Nma) GV1YAFPK (Dnp) rr	1591. 77	1590. 8055
YYAFPK (Dnp) rr	1266. 36	1265. 5942	GVPYAFPK (Dnp) rr	1356. 49	1355. 6735	RYYAFPK (Dnp) rr	1422. 55	1421. 6953	A2pr (Nma) GVDYAFPK (Dnp) rr	1593. 70	1592. 7484
APYAFPK (Dnp) rr	1271. 38	1270. 6207	RPYAFPK (Dnp) rr	1356. 49	1355. 6847	GR1YAFPK (Dnp) rr	1429. 58	1428. 7375	A2pr (Nma) GEPYAFPK (Dnp) rr	1605. 71	1604. 7484
A2pr (Nma) GV1YAFPK (Dnp)	1279. 40	1278. 6033	A2pr (Nma) GEYAFPK (Dnp)	1359. 40	1358. 5568	GRDYAFPK (Dnp) rr	1431. 51	1430. 6803	A2pr (Nma) GVKYAFPK (Dnp) rr	1606. 78	1605. 8164
A2pr (Nma) GVDYAFPK (Dnp)	1281. 33	1280. 5462	GAKYAFPK (Dnp) rr	1359. 49	1358. 6844	A2pr (Nma) GV1YAFPK (Dnp) r	1435. 58	1434. 7044	A2pr (Nma) GAYYAFPK (Dnp) rr	1613. 73	1612. 7535
AIYAFPK (Dnp) rr	1287. 43	1286. 6520	EKYAFPK (Dnp) rr	1360. 48	1359. 6684	GFKYAFPK (Dnp) rr	1435. 59	1434. 7157	A2pr (Nma) GE1YAFPK (Dnp) rr	1621. 75	1620. 7797
ADYAFPK (Dnp) rr	1289. 36	1288. 5949	F1YAFPK (Dnp) rr	1363. 52	1362. 6833	A2pr (Nma) GVDYAFPK (Dnp) r	1437. 51	1436. 6473	A2pr (Nma) GEDYAFPK (Dnp) rr	1623. 68	1622. 7226
A2pr (Nma) GEPYAFPK (Dnp)	1293. 34	1292. 5462	FDYAFPK (Dnp) rr	1365. 45	1364. 6262	GRKYAFPK (Dnp) rr	1444. 60	1443. 7484	A2pr (Nma) GFPYAFPK (Dnp) rr	1623. 77	1622. 7742
A2pr (Nma) GVKYAFPK (Dnp)	1294. 41	1293. 6142	VYYAFPK (Dnp) rr	1365. 49	1364. 6626	A2pr (Nma) GEPYAFPK (Dnp) r	1449. 52	1448. 6473	A2pr (Nma) GRPYAFPK (Dnp) rr	1632. 78	1631. 8069
VYAFPK (Dnp) rr	1299. 44	1298. 6520	GV1YAFPK (Dnp) rr	1372. 53	1371. 7048	A2pr (Nma) GVKYAFPK (Dnp) r	1450. 60	1449. 7153	A2pr (Nma) GEKYAFPK (Dnp) rr	1636. 77	1635. 7906
A2pr (Nma) GAYYAFPK (Dnp)	1301. 36	1300. 5513	R1YAFPK (Dnp) rr	1372. 53	1371. 7160	GEYYAFPK (Dnp) rr	1452. 53	1451. 6582	A2pr (Nma) GF1YAFPK (Dnp) rr	1639. 81	1638. 8055
AKYAFPK (Dnp) rr	1302. 44	1301. 6629	GVDYAFPK (Dnp) rr	1374. 46	1373. 6476	A2pr (Nma) GAYYAFPK (Dnp) r	1457. 55	1456. 6524	A2pr (Nma) GFDYAFPK (Dnp) rr	1641. 74	1640. 7484
A2pr (Nma) GE1YAFPK (Dnp)	1309. 38	1308. 5775	RDYAFPK (Dnp) rr	1374. 46	1373. 6589	A2pr (Nma) GE1YAFPK (Dnp) r	1465. 57	1464. 6786	A2pr (Nma) GVYYAFPK (Dnp) rr	1641. 79	1640. 7848
A2pr (Nma) GEDYAFPK (Dnp)	1311. 31	1310. 5204	A2pr (Nma) GFYYAFPK (Dnp)	1377. 46	1376. 5826	A2pr (Nma) GEDYAFPK (Dnp) r	1467. 50	1466. 6215	A2pr (Nma) GR1YAFPK (Dnp) rr	1648. 82	1647. 8382
A2pr (Nma) GFPYAFPK (Dnp)	1311. 40	1310. 5720	FKYAFPK (Dnp) rr	1378. 54	1377. 6942	A2pr (Nma) GFPYAFPK (Dnp) r	1467. 58	1466. 6731	A2pr (Nma) GRDYAFPK (Dnp) rr	1650. 75	1649. 7811
V1YAFPK (Dnp) rr	1315. 48	1314. 6833	A2pr (Nma) GRYYAFPK (Dnp)	1386. 47	1385. 6153	GFYYAFPK (Dnp) rr	1470. 59	1469. 6840	A2pr (Nma) GFKYAFPK (Dnp) rr	1654. 83	1653. 8164
VDYAFPK (Dnp) rr	1317. 41	1316. 6262	GEPYAFPK (Dnp) rr	1386. 47	1385. 6476	A2pr (Nma) GRPYAFPK (Dnp) r	1476. 60	1475. 7058	A2pr (Nma) GRKYAFPK (Dnp) rr	1663. 84	1662. 8491
A2pr (Nma) GRPYAFPK (Dnp)	1320. 41	1319. 6047	GVKYAFPK (Dnp) rr	1387. 54	1386. 7157	GRYYAFPK (Dnp) rr	1479. 60	1478. 7167	A2pr (Nma) GEYYAFPK (Dnp) rr	1671. 77	1670. 7590
A2pr (Nma) GEKYAFPK (Dnp)	1324. 40	1323. 5884	RKYAFPK (Dnp) rr	1387. 55	1386. 7269	A2pr (Nma) GEKYAFPK (Dnp) r	1480. 58	1479. 6895	A2pr (Nma) GFYYAFPK (Dnp) rr	1689. 83	1688. 7848
A2pr (Nma) GF1YAFPK (Dnp)	1327. 44	1326. 6033	A2pr (Nma) GAPYAFPK (Dnp) r	1391. 49	1390. 6418	A2pr (Nma) GF1YAFPK (Dnp) r	1483. 63	1482. 7044	A2pr (Nma) GRYYAFPK (Dnp) rr	1698. 84	1697. 8175
GAPYAFPK (Dnp) rr	1328. 43	1327. 6422	GAYYAFPK (Dnp) rr	1394. 49	1393. 6527	A2pr (Nma) GFDYAFPK (Dnp) r	1485. 56	1484. 6473			
A2pr (Nma) GFDYAFPK (Dnp)	1329. 37	1328. 5462	EYYAFPK (Dnp) rr	1395. 48	1394. 6367	A2pr (Nma) GVYYAFPK (Dnp) r	1485. 60	1484. 6837			
A2pr (Nma) GVYYAFPK (Dnp)	1329. 41	1328. 5826	GE1YAFPK (Dnp) rr	1402. 51	1401. 6789	A2pr (Nma) GR1YAFPK (Dnp) r	1492. 64	1491. 7371			
EPYAFPK (Dnp) rr	1329. 42	1328. 6262	GEDYAFPK (Dnp) rr	1404. 44	1403. 6218	A2pr (Nma) GRDYAFPK (Dnp) r	1494. 57	1493. 6800			
YKYAFPK (Dnp) rr	1330. 49	1329. 6942	GFPYAFPK (Dnp) rr	1404. 53	1403. 6735	A2pr (Nma) GFKYAFPK (Dnp) r	1498. 64	1497. 7153			