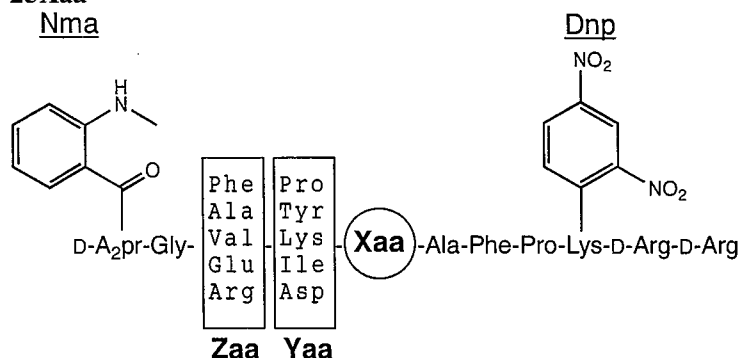


FRETs-25Xaa Series

* 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 $\lambda_{\text{ex}} = 340 \text{ nm}$ and $\lambda_{\text{em}} = 440 \text{ nm}$ 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 μl of each of the above substrate stock solution with 1980 μl of an appropriate buffer (10 μM)

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

- 1) Set a fluorescence spectrophotometer at $\lambda_{\text{ex}} = 340 \text{ nm}$ and $\lambda_{\text{em}} = 440 \text{ nm}$
- 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 μ 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 μ l of the stock solution of the best Xaa-containing substrate chosen by the above primary screening with 1800 μ l of an appropriate buffer (100 μ M)

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

- 1) Set a fluorescence spectrophotometer at $\lambda_{ex} = 340$ nm and $\lambda_{em} = 440$ 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 μ 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 μ l aliquots to LC-MS

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

·Analytical conditions

column: ODS

eluant: A) H₂O containing 0.05% TFA, B) CH₃CN 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 μ 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-25Gln	Average	Monoisotopic	FRETs-25Gln	Average	Monoisotopic	FRETs-25Gln	Average	Monoisotopic	FRETs-25Gln	Average	Monoisotopic
A2pr (Nma) G	294. 31	294. 1328	A2pr (Nma) GVPQ	618. 68	618. 3126	A2pr (Nma) GE1QA	735. 79	735. 3552	A2pr (Nma) GYQAF	902. 99	902. 4287
A2pr (Nma) GA	365. 38	365. 1699	A2pr (Nma) GAKQ	621. 69	621. 3235	A2pr (Nma) GEDQA	737. 71	737. 2980	A2pr (Nma) GAPQAFP	906. 00	905. 4396
A2pr (Nma) GV	393. 44	393. 2012	K (Dnp) rr	624. 65	624. 3092	A2pr (Nma) GFPQA	737. 80	737. 3497	A2pr (Nma) GR1QAF	910. 03	909. 4821
A2pr (Nma) GE	423. 42	423. 1754	A2pr (Nma) GV1Q	634. 72	634. 3439	A2pr (Nma) GRYQ	741. 79	741. 3558	A2pr (Nma) GRDQAF	911. 96	911. 4250
A2pr (Nma) GF	441. 48	441. 2012	A2pr (Nma) GVDQ	636. 65	636. 2867	A2pr (Nma) GRPQA	746. 81	746. 3824	A2pr (Nma) GFKQAF	916. 03	915. 4603
A2pr (Nma) GR	450. 49	450. 2339	A2pr (Nma) GEPQ	648. 66	648. 2867	A2pr (Nma) GEKQA	750. 80	750. 3661	A2pr (Nma) GA1QAFP	922. 04	921. 4709
A2pr (Nma) GAP	462. 50	462. 2227	A2pr (Nma) GVKQ	649. 74	649. 3548	A2pr (Nma) GF1QA	753. 85	753. 3810	A2pr (Nma) GADQAFP	923. 97	923. 4137
A2pr (Nma) GA1	478. 54	478. 2540	A2pr (Nma) GAYQ	656. 69	656. 2918	A2pr (Nma) GFDQA	755. 77	755. 3239	A2pr (Nma) GRKQAF	925. 05	924. 4930
A2pr (Nma) GAD	480. 47	480. 1969	A2pr (Nma) GAPQA	661. 71	661. 3184	A2pr (Nma) GYVQA	755. 82	755. 3602	A2pr (Nma) GEYQAF	932. 97	932. 4028
A2pr (Nma) GVP	490. 55	490. 2540	A2pr (Nma) GE1Q	664. 71	664. 3180	A2pr (Nma) GR1QA	762. 86	762. 4137	A2pr (Nma) GVPQAFP	934. 05	933. 4709
A2pr (Nma) GAK	493. 56	493. 2649	A2pr (Nma) GEDQ	666. 64	666. 2609	A2pr (Nma) GRDQA	764. 79	764. 3566	A2pr (Nma) GAKQAFP	937. 05	936. 4818
A2pr (Nma) GVI	506. 60	506. 2853	Ac-K (Dnp) rr	666. 69	666. 3126	A2pr (Nma) GFKQA	768. 86	768. 3919	AFPK (Dnp) rr	940. 02	939. 4675
A2pr (Nma) GVD	508. 52	508. 2282	A2pr (Nma) GFPQ	666. 72	666. 3198	A2pr (Nma) GRKQA	777. 87	777. 4246	A2pr (Nma) GV1QAFP	950. 09	949. 5022
A2pr (Nma) GEP	520. 54	520. 2282	A2pr (Nma) GRPQ	675. 74	675. 3453	A2pr (Nma) GEYQA	785. 80	785. 3344	A2pr (Nma) GFYQAF	951. 03	950. 4287
A2pr (Nma) GVK	521. 61	521. 2962	A2pr (Nma) GA1QA	677. 75	677. 3497	A2pr (Nma) GFYQA	803. 86	803. 3602	A2pr (Nma) GVDQAFP	952. 02	951. 4450
A2pr (Nma) GAY	528. 56	528. 2332	A2pr (Nma) GADQA	679. 68	679. 2926	A2pr (Nma) GAPQAF	808. 88	808. 3868	A2pr (Nma) GRYQAF	960. 05	959. 4613
A2pr (Nma) GE1	536. 58	536. 2595	A2pr (Nma) GEKQ	679. 72	679. 3289	A2pr (Nma) GRYQA	812. 87	812. 3929	A2pr (Nma) GEPQAFP	964. 03	963. 4450
A2pr (Nma) GED	538. 51	538. 2023	A2pr (Nma) GF1Q	682. 77	682. 3439	A2pr (Nma) GA1QAF	824. 92	824. 4181	A2pr (Nma) GVKQAFP	965. 11	964. 5131
A2pr (Nma) GFP	538. 60	538. 2540	A2pr (Nma) GFDQ	684. 70	684. 2867	A2pr (Nma) GADQAF	826. 85	826. 3610	A2pr (Nma) GAYQAFP	972. 05	971. 4501
A2pr (Nma) GRP	547. 61	547. 2867	A2pr (Nma) GYVQ	684. 74	684. 3231	A2pr (Nma) GVPQAF	836. 93	836. 4181	A2pr (Nma) GE1QAFP	980. 07	979. 4763
A2pr (Nma) GEK	551. 59	551. 2704	A2pr (Nma) GVPQA	689. 76	689. 3497	A2pr (Nma) GAKQAF	839. 94	839. 4290	A2pr (Nma) GEDQAFP	982. 00	981. 4192
A2pr (Nma) GF1	554. 64	554. 2853	A2pr (Nma) GR1Q	691. 78	691. 3766	A2pr (Nma) GV1QAF	852. 98	852. 4494	A2pr (Nma) GFPQAFP	982. 09	981. 4709
A2pr (Nma) GFD	556. 57	556. 2282	A2pr (Nma) GAKQA	692. 76	692. 3606	A2pr (Nma) GVDQAF	854. 91	854. 3923	A2pr (Nma) GRPQAFP	991. 10	990. 5035
A2pr (Nma) GYV	556. 61	556. 2645	A2pr (Nma) GRDQ	693. 71	693. 3194	A2pr (Nma) GEPQAF	866. 92	866. 3923	A2pr (Nma) GEKQAFP	995. 09	994. 4872
A2pr (Nma) GR1	563. 65	563. 3180	A2pr (Nma) GFKQ	697. 78	697. 3548	A2pr (Nma) GVKQAF	867. 99	867. 4603	A2pr (Nma) GF1QAFP	998. 13	997. 5022
A2pr (Nma) GRD	565. 58	565. 2609	A2pr (Nma) GV1QA	705. 80	705. 3810	FPK (Dnp) rr	868. 94	868. 4304	A2pr (Nma) GFDQAFP	1000. 06	999. 4450
A2pr (Nma) GFK	569. 65	569. 2962	A2pr (Nma) GRKQ	706. 79	706. 3875	A2pr (Nma) GAYQAF	874. 94	874. 3974	A2pr (Nma) GYVQAFP	1000. 11	999. 4814
A2pr (Nma) GRK	578. 66	578. 3289	A2pr (Nma) GVDQA	707. 73	707. 3239	A2pr (Nma) GE1QAF	882. 96	882. 4236	A2pr (Nma) GR1QAFP	1007. 15	1006. 5348
A2pr (Nma) GEY	586. 59	586. 2387	A2pr (Nma) GEYQ	714. 72	714. 2973	A2pr (Nma) GEDQAF	884. 89	884. 3664	A2pr (Nma) GRDQAFP	1009. 08	1008. 4777
A2pr (Nma) GAPQ	590. 63	590. 2813	A2pr (Nma) GEPQA	719. 74	719. 3239	A2pr (Nma) GFPQAF	884. 98	884. 4181	A2pr (Nma) GFKQAFP	1013. 15	1012. 5131
A2pr (Nma) GFY	604. 65	604. 2645	A2pr (Nma) GVKQA	720. 82	720. 3919	A2pr (Nma) GRPQAF	893. 99	893. 4508	A2pr (Nma) GRKQAFP	1022. 16	1021. 5457
A2pr (Nma) GA1Q	606. 67	606. 3126	PK (Dnp) rr	721. 77	721. 3620	A2pr (Nma) GEKQAF	897. 97	897. 4345	A2pr (Nma) GEYQAFP	1030. 09	1029. 4556
A2pr (Nma) GADQ	608. 60	608. 2554	A2pr (Nma) GAYQA	727. 76	727. 3289	A2pr (Nma) GF1QAF	901. 02	900. 4494	A2pr (Nma) GFYQAFP	1048. 15	1047. 4814
A2pr (Nma) GRY	613. 67	613. 2972	A2pr (Nma) GFYQ	732. 78	732. 3231	A2pr (Nma) GFDQAF	902. 95	902. 3923	A2pr (Nma) GRYQAFP	1057. 16	1056. 5141

FRETs-25Gln	Average	Monoisotopic	FRETs-25Gln	Average	Monoisotopic	FRETs-25Gln	Average	Monoisotopic	FRETs-25Gln	Average	Monoisotopic
QAAPK (Dnp) rr	1068. 15	1067. 5261	A2pr (Nma) GRQAAPK (Dnp)	1301. 41	1300. 6313	A2pr (Nma) GAQAAPK (Dnp) r	1372. 49	1371. 6684	A2pr (Nma) GRQAAPK (Dnp) r	1472. 61	1471. 7433
PQAAPK (Dnp) rr	1165. 26	1164. 5788	AYQAAPK (Dnp) rr	1302. 40	1301. 6265	A2pr (Nma) GADQAAPK (Dnp) r	1374. 42	1373. 6113	A2pr (Nma) GEYQAAPK (Dnp) r	1480. 54	1479. 6531
IQQAAPK (Dnp) rr	1181. 30	1180. 6101	A2pr (Nma) GRDQAAPK (Dnp)	1303. 34	1302. 5741	FYQAAPK (Dnp) rr	1378. 49	1377. 6578	A2pr (Nma) GFYQAAPK (Dnp) r	1498. 60	1497. 6789
DQAAPK (Dnp) rr	1183. 23	1182. 5530	A2pr (Nma) GFKQAAPK (Dnp)	1307. 41	1306. 6095	GRPQAAPK (Dnp) rr	1378. 50	1377. 7014	A2pr (Nma) GRYQAAPK (Dnp) r	1507. 61	1506. 7116
KQAAPK (Dnp) rr	1196. 32	1195. 6210	GAQAAPK (Dnp) rr	1309. 43	1308. 6687	GEKQAAPK (Dnp) rr	1382. 48	1381. 6851	A2pr (Nma) GAPQAAPK (Dnp) rr	1512. 63	1511. 7382
A2pr (Nma) GAPQAAPK (Dnp)	1200. 26	1199. 5360	EIQQAAPK (Dnp) rr	1310. 42	1309. 6527	A2pr (Nma) GVPQAAPK (Dnp) r	1384. 50	1383. 6684	A2pr (Nma) GAQAAPK (Dnp) rr	1528. 67	1527. 7695
A2pr (Nma) GAQAAPK (Dnp)	1216. 30	1215. 5673	GADQAAPK (Dnp) rr	1311. 36	1310. 6116	GFQAAPK (Dnp) rr	1385. 53	1384. 7000	A2pr (Nma) GADQAAPK (Dnp) rr	1530. 60	1529. 7124
A2pr (Nma) GADQAAPK (Dnp)	1218. 23	1217. 5102	EDQAAPK (Dnp) rr	1312. 35	1311. 5956	GFQAAPK (Dnp) rr	1387. 46	1386. 6429	A2pr (Nma) GVPQAAPK (Dnp) rr	1540. 68	1539. 7695
A2pr (Nma) GVPQAAPK (Dnp)	1228. 31	1227. 5673	FPQAAPK (Dnp) rr	1312. 44	1311. 6473	A2pr (Nma) GAKQAAPK (Dnp) r	1387. 50	1386. 6793	A2pr (Nma) GAKQAAPK (Dnp) rr	1543. 69	1542. 7804
A2pr (Nma) GAKQAAPK (Dnp)	1231. 32	1230. 5782	A2pr (Nma) GRKQAAPK (Dnp)	1316. 42	1315. 6422	GVYQAAPK (Dnp) rr	1387. 50	1386. 6793	A2pr (Nma) GVIQAAPK (Dnp) rr	1556. 73	1555. 8008
YQAAPK (Dnp) rr	1231. 32	1230. 5894	GVPQAAPK (Dnp) rr	1321. 44	1320. 6687	RYQAAPK (Dnp) rr	1387. 51	1386. 6905	A2pr (Nma) GVDQAAPK (Dnp) rr	1558. 66	1557. 7437
APQAAPK (Dnp) rr	1236. 34	1235. 6160	RPQAAPK (Dnp) rr	1321. 45	1320. 6800	GRQAAPK (Dnp) rr	1394. 54	1393. 7327	A2pr (Nma) GEPQAAPK (Dnp) rr	1570. 67	1569. 7437
A2pr (Nma) GVIQAAPK (Dnp)	1244. 35	1243. 5986	A2pr (Nma) GEYQAAPK (Dnp)	1324. 35	1323. 5520	GRDQAAPK (Dnp) rr	1396. 47	1395. 6756	A2pr (Nma) GVKQAAPK (Dnp) rr	1571. 74	1570. 8117
A2pr (Nma) GVDQAAPK (Dnp)	1246. 28	1245. 5415	GAKQAAPK (Dnp) rr	1324. 45	1323. 6796	A2pr (Nma) GVIQAAPK (Dnp) r	1400. 54	1399. 6997	A2pr (Nma) GAYQAAPK (Dnp) rr	1578. 69	1577. 7488
AIQAAPK (Dnp) rr	1252. 38	1251. 6473	EKQAAPK (Dnp) rr	1325. 43	1324. 6636	GFKQAAPK (Dnp) rr	1400. 54	1399. 7109	A2pr (Nma) GEIQAAPK (Dnp) rr	1586. 71	1585. 7750
ADQAAPK (Dnp) rr	1254. 31	1253. 5901	FIQAAPK (Dnp) rr	1328. 48	1327. 6786	A2pr (Nma) GVDQAAPK (Dnp) r	1402. 47	1401. 6426	A2pr (Nma) GEDQAAPK (Dnp) rr	1588. 64	1587. 7179
A2pr (Nma) GEPQAAPK (Dnp)	1258. 30	1257. 5415	FDQAAPK (Dnp) rr	1330. 41	1329. 6214	GRKQAAPK (Dnp) rr	1409. 56	1408. 7436	A2pr (Nma) GFPQAAPK (Dnp) rr	1588. 73	1587. 7695
A2pr (Nma) GVKQAAPK (Dnp)	1259. 37	1258. 6095	YYQAAPK (Dnp) rr	1330. 45	1329. 6578	A2pr (Nma) GEPQAAPK (Dnp) r	1414. 48	1413. 8426	A2pr (Nma) GRPQAAPK (Dnp) rr	1597. 74	1596. 8022
VPQAAPK (Dnp) rr	1264. 39	1263. 6473	GVIQAAPK (Dnp) rr	1337. 49	1336. 7000	A2pr (Nma) GVKQAAPK (Dnp) r	1415. 55	1414. 7106	A2pr (Nma) GEKQAAPK (Dnp) rr	1601. 72	1600. 7859
A2pr (Nma) GAYQAAPK (Dnp)	1266. 32	1265. 5465	RIQAAPK (Dnp) rr	1337. 49	1336. 7113	GEYQAAPK (Dnp) rr	1417. 48	1416. 6535	A2pr (Nma) GFIQAAPK (Dnp) rr	1604. 77	1603. 8008
AKQAAPK (Dnp) rr	1267. 40	1266. 6582	GVDQAAPK (Dnp) rr	1339. 42	1338. 6429	A2pr (Nma) GAYQAAPK (Dnp) r	1422. 50	1421. 6476	A2pr (Nma) GFDQAAPK (Dnp) rr	1606. 70	1605. 7437
A2pr (Nma) GEIQAAPK (Dnp)	1274. 34	1273. 5728	RDQAAPK (Dnp) rr	1339. 42	1338. 6541	A2pr (Nma) GEIQAAPK (Dnp) r	1430. 52	1429. 6739	A2pr (Nma) GVIQAAPK (Dnp) rr	1606. 74	1605. 7801
A2pr (Nma) GEDQAAPK (Dnp)	1276. 27	1275. 5156	A2pr (Nma) GFYQAAPK (Dnp)	1342. 41	1341. 5778	A2pr (Nma) GEDQAAPK (Dnp) r	1432. 45	1431. 6167	A2pr (Nma) GRQAAPK (Dnp) rr	1613. 78	1612. 8335
A2pr (Nma) GFPQAAPK (Dnp)	1276. 35	1275. 5673	FKQAAPK (Dnp) rr	1343. 49	1342. 6895	A2pr (Nma) GFPQAAPK (Dnp) r	1432. 54	1431. 6684	A2pr (Nma) GRDQAAPK (Dnp) rr	1615. 71	1614. 7764
VIQAAPK (Dnp) rr	1280. 43	1279. 6786	A2pr (Nma) GRYQAAPK (Dnp)	1351. 42	1350. 6105	GFYQAAPK (Dnp) rr	1435. 54	1434. 6793	A2pr (Nma) GFKQAAPK (Dnp) rr	1619. 78	1618. 8117
VDQAAPK (Dnp) rr	1282. 36	1281. 6214	GEPQAAPK (Dnp) rr	1351. 43	1350. 6429	A2pr (Nma) GRPQAAPK (Dnp) r	1441. 55	1440. 7011	A2pr (Nma) GRKQAAPK (Dnp) rr	1628. 80	1627. 8444
A2pr (Nma) GRPQAAPK (Dnp)	1285. 37	1284. 6000	GVKQAAPK (Dnp) rr	1352. 50	1351. 7109	GRYQAAPK (Dnp) rr	1444. 56	1443. 7120	A2pr (Nma) GEYQAAPK (Dnp) rr	1636. 72	1635. 7542
A2pr (Nma) GEKQAAPK (Dnp)	1289. 35	1288. 5836	RKQAAPK (Dnp) rr	1352. 50	1351. 7221	A2pr (Nma) GEKQAAPK (Dnp) r	1445. 54	1444. 6848	A2pr (Nma) GFYQAAPK (Dnp) rr	1654. 78	1653. 7801
A2pr (Nma) GFIQAAPK (Dnp)	1292. 40	1291. 5986	A2pr (Nma) GAPQAAPK (Dnp) r	1356. 44	1355. 6371	A2pr (Nma) GFIQAAPK (Dnp) r	1448. 58	1447. 6997	A2pr (Nma) GRYQAAPK (Dnp) rr	1663. 80	1662. 8128
GAPQAAPK (Dnp) rr	1293. 39	1292. 6374	GAYQAAPK (Dnp) rr	1359. 45	1358. 6480	A2pr (Nma) GFDQAAPK (Dnp) r	1450. 51	1449. 6426			
A2pr (Nma) GFDQAAPK (Dnp)	1294. 33	1293. 5415	EYQAAPK (Dnp) rr	1360. 43	1359. 6320	A2pr (Nma) GVIQAAPK (Dnp) r	1450. 56	1449. 6789			
A2pr (Nma) GVIQAAPK (Dnp)	1294. 37	1293. 5778	GEIQAAPK (Dnp) rr	1367. 47	1366. 6742	A2pr (Nma) GRQAAPK (Dnp) r	1457. 59	1456. 7324			
EPQAAPK (Dnp) rr	1294. 38	1293. 6214	GEDQAAPK (Dnp) rr	1369. 40	1368. 6171	A2pr (Nma) GRDQAAPK (Dnp) r	1459. 52	1458. 6753			
YKQAAPK (Dnp) rr	1295. 45	1294. 6895	GFPQAAPK (Dnp) rr	1369. 49	1368. 6687	A2pr (Nma) GFKQAAPK (Dnp) r	1463. 60	1462. 7106			