

Figure 1: Inhibition of LAD2 cells by disodium cromoglycate. Four different concentrations of DSCG were investigated, with application to cells 2 minutes before activation. Cells were activated with human IgE-biotin conjugate/streptavidin (in 1:1 ratio) at 500 ng/ml.

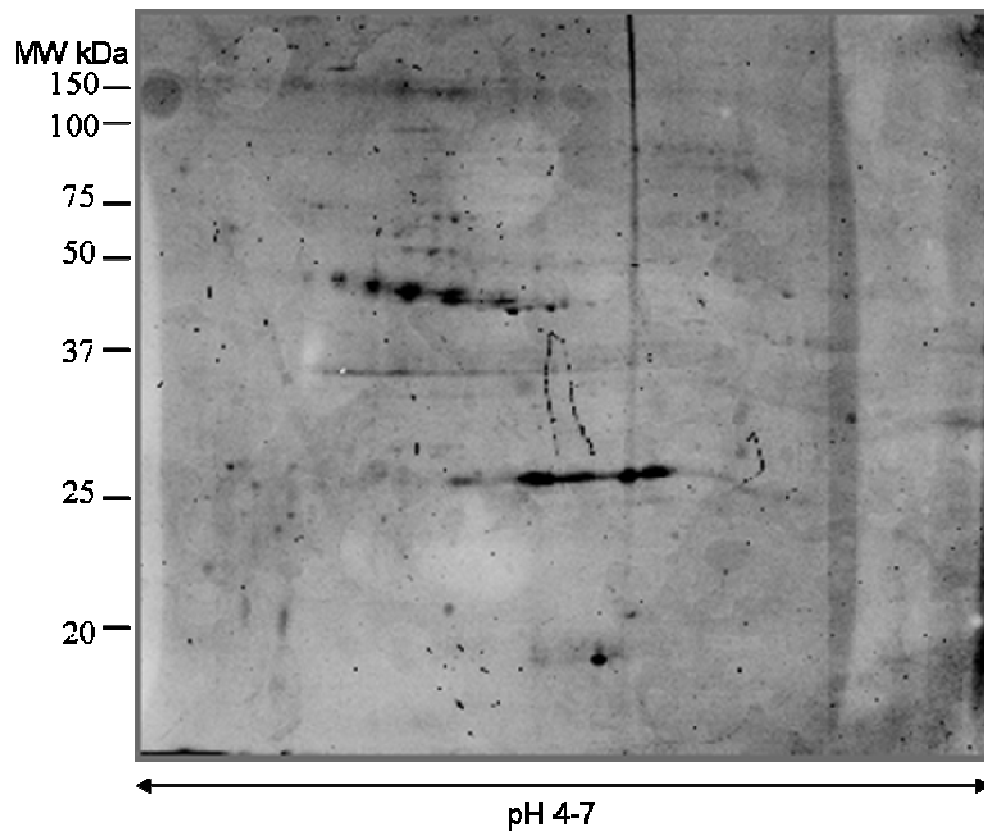


Figure 2: Control gel image of representative 2-DE gel of LAD2 membrane fraction stained with Pro-Q® Diamond phosphoprotein stain.

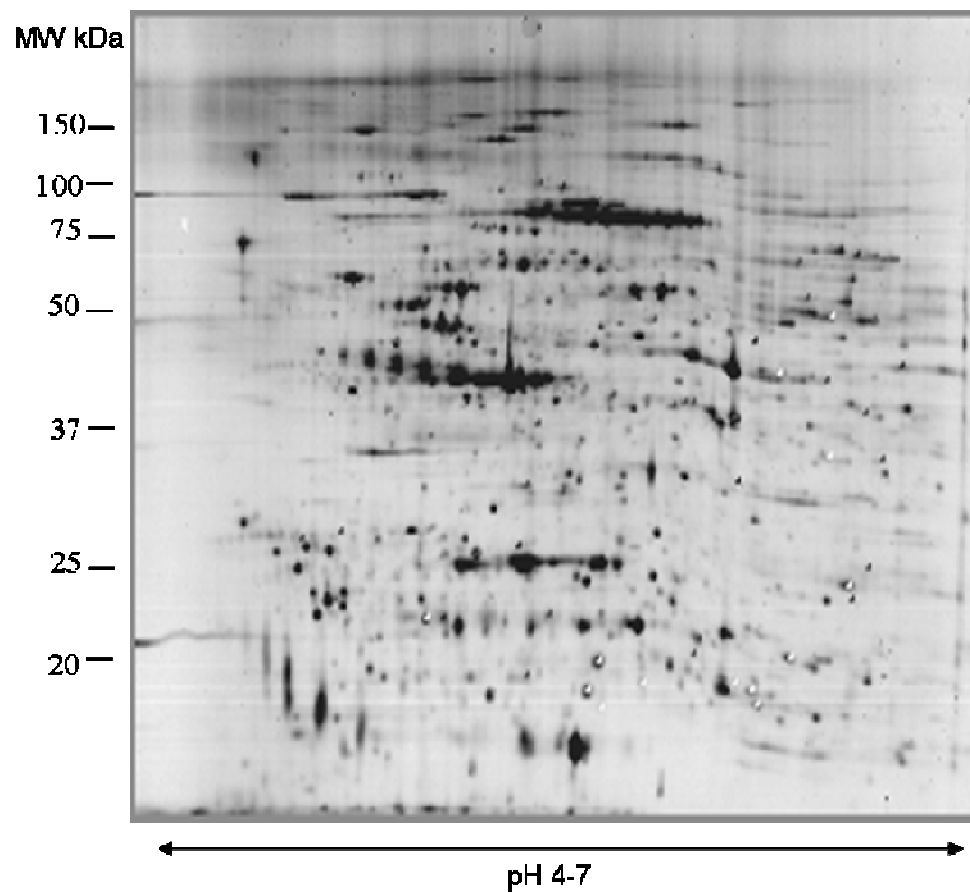


Figure 3: Control gel image of representative 2-DE gel of LAD2 membrane fraction stained with SYPRO® Ruby, after stimulation of cells with IgE.

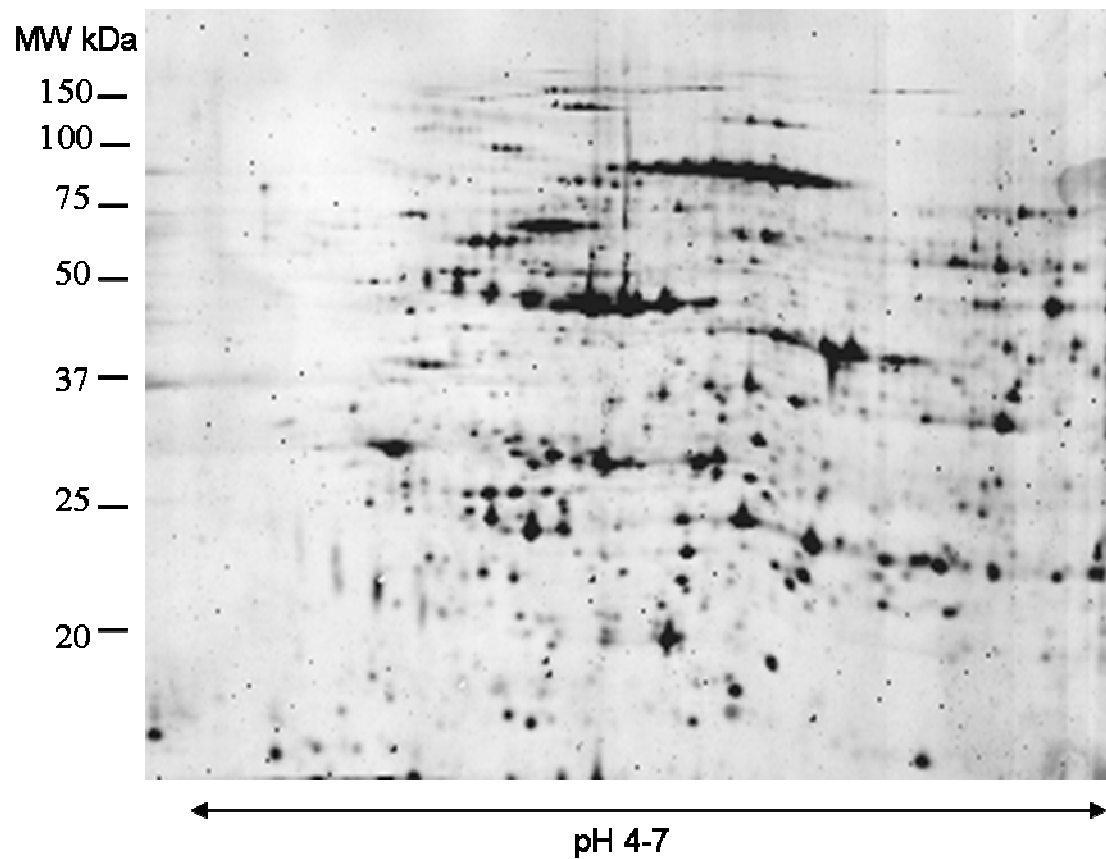


Figure 4: Control gel image of representative 2-DE gel of LAD2 soluble fraction stained with SYPRO® Ruby, after stimulation of cells with IgE.

### **MALDI Peak List Data**

Spectra were collected and processed as described in Methods section of paper. Frequently-observed trypsin and keratin peaks were excluded in the generation of peak lists of monoisotopic ions. Peaks lists were used to search the SwissProt database using the Mascot search engine ([www.matrixscience.com](http://www.matrixscience.com)). The data presented below provide details of peak masses, search parameters and proteins identified.

**Table 1: Proteins showing increased phosphorylation upon stimulation.**

Peptide mass lists are tabulated below, along with Mascot search summary for each protein spot.

MALDI plate and spot number

SwissProt accession number, SwissProt code, protein name

[MOWSE score, expect value, matching peptides/total peptides, percentage coverage; theoretical MW, pI]

Scores in red indicate a statistically significant identification ( $p < 0.05$ ) from Mascot search.

**Plate 88**

Gel stain: ProQ Diamond phosphostain and SYPRO Ruby protein stain

Database: SwissProt 48.6 (259907 sequences) – 16 Dec 2005

Taxonomy: unrestricted

Error tolerance: 50 ppm

Variable modifications: CAM-C, Met-ox, prop-C

Any deviations from these parameters are indicated adjacent to spot number.

**88-G7**

**P13796 (PLSL\_HUMAN) L-plastin**

**[185 8.2e-14 17/23 31%; 70.2K 5.20]**

917.384	942.524	994.513	998.516	1012.589	1069.592
1117.565	1118.570	1135.586	1154.572	1186.589	1303.627
1319.612	1335.616	1431.752	1459.655	1518.727	1534.741
1537.765	1585.828	1675.822	1759.847	1893.850	

**88-H7**

**P13796 (PLSL\_HUMAN) L-plastin**

**[105 8.2e-06 14/33 26%; 70.2K 5.20]**

908.960	942.547	945.556	976.470	994.536	998.525
1069.624	1082.052	1098.027	1117.576	1118.579	1132.529
1135.609	1186.550	1251.542	1303.611	1319.618	1335.599
1365.650	1414.560	1490.630	1499.675	1516.686	1518.736
1585.826	1675.826	1700.743	1759.849	1775.826	1790.863
1875.801	1893.845	1954.050			

**88-D7 (Taxonomy: human)**

**P07437 (TBB2C\_HUMAN) Tubulin beta-2C chain**

**[153 2.8e-11 15/29 35%; 49.8K 4.79]**

1028.526	1039.593	1063.519	1069.603	1077.541	1130.595
1159.635	1175.620	1245.602	1261.595	1287.730	1301.650
1328.662	1335.715	1397.686	1401.690	1406.688	1435.766
1462.713	1617.821	1631.846	1647.832	1659.901	1701.916
1707.868	1718.895	1728.845	1873.929	1959.001	

**88-E7 (Taxonomy: human; error tolerance: 100 ppm)**

**P07437 (TBB2\_HUMAN) Tubulin beta-2 chain**

**[125 1.8e-08 9/10 25%; 49.6K 4.78]**

1039.642	1077.595	1130.655	1159.716	1245.662	1301.718
1328.743	1335.786	1631.887	1659.976		

**88-G6**

**P06748 (NPM\_HUMAN) Nucleophosmin**

**[84 0.0011 6/9 22%; 32.6K 4.64]**

910.429	931.486	974.518	1023.514	1558.720	1568.741
1582.756	1603.767	2161.022			

**Table 2: Proteins recruited to the membrane upon stimulation.**

Peptide mass lists are tabulated below, along with Mascot search summary for each protein spot.

Gel position number: MALDI plate and spot number

SwissProt accession number, SwissProt code, protein name

[MOWSE score, expect value, matching peptides/total peptides, percentage coverage; theoretical MW, pI]

Scores in red indicate a statistically significant identification ( $p < 0.05$ ) from Mascot search.

**Plate 75**

Protein stain: silver

Database: SwissProt 47.6 (243668 sequences) – 18 Aug 2005 or

SwissProt 47.7 (245699 sequences) (human 53508 sequences) – 22 Aug 2005

Taxonomy: unrestricted

Error tolerance: 100 ppm

Variable modifications: CAM-Cys, Met-Ox

Any deviations from these parameters are indicated adjacent to spot number.

**1: 75-A5 (Database: SwissProt 47.7; taxonomy: human; variable modifications: + prop-C)  
P28331 (NDUS1\_HUMAN) NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial  
precursor**

**[90 6.0e-05 8/9 14%; 79.4K pI 5.89]**

911.329	994.144	1064.553	1225.166	1401.754	1425.749
1491.818	1607.747	1626.756	1644.789	1839.967	

**2: 75-A1 (Taxonomy: human; error tolerance: 75 ppm)**

**P13796 (PLSL\_HUMAN) L-plastin**

**[113 2.7e-07 11/18 21%; 70.2K pI 5.20]**

998.552	1011.656	1051.700	1069.638	1117.604	1135.629
1338.853	1405.827	1431.772	1518.768	1534.836	1585.855
1666.008	1675.862	1759.870	1803.942	1831.935	1885.038

**3: 75-C2 (Database: SwissProt 47.7; taxonomy: human; variable modifications: + prop-C)**

**P30101 (PDIA3\_HUMAN) Protein disulfide-isomerase A3 precursor**

**[55 0.74 7/13 11%; 56.7K pI 5.98]**

1172.568	1188.557	1341.692	1359.673	1370.713	1437.765
1475.704	1515.788	1579.879	1596.932	1782.793	1794.813
2036.004					

**11: 75-A3**

**P30740 (ILEU\_HUMAN) Leukocyte elastase inhibitor**

**[161 1.9e-11 15/28 34%; 42.7K pI 5.90]**

1006.510	1102.611	1126.568	1149.597	1178.580	1179.728
1194.558	1207.651	1218.615	1230.704	1287.705	1305.713
1333.715	1346.716	1389.778	1496.877	1510.760	1602.784
1650.800	1686.870	1785.914	1809.803	1860.907	1896.030
1934.868	1940.931	2003.061	2035.957		

**16: 75-H3**

**P15428 (PGDH\_HUMAN) 15-hydroxyprostaglandin dehydrogenase [NAD+]**

**[68 0.04 6/17 30%; 29.0K pI 5.56]**

1030.601	1095.679	1212.727	1275.642	1390.726	1421.908
1512.805	1767.829	1798.800	1809.800	1826.800	1834.915
1977.005	2008.985	2018.972	2035.993	2044.090	

**18: 75-D7 (Database: SwissProt 47.7)**

**P07203 (GPX1\_HUMAN) Glutathione peroxidase 1**

**[66 0.068 5/13 28 %; 21.9K pI 6.15]**

1154.513	1163.596	1169.675	1313.590	1325.779	1352.770
1496.802	1496.802	1653.958	1741.870	1758.873	1798.797
2115.034					

**21: 75-H1 (Taxonomy: human; error tolerance: 75 ppm)**

**P61224 (RAP1B\_HUMAN) Ras-related protein Rap-1B**

**[76 0.0013 7/25 45 %; 20.8K pI 5.65]**

915.459	985.634	1011.668	1018.792	1065.719	1191.686
1208.630	1220.837	1254.771	1258.595	1314.803	1332.756
1338.816	1366.873	1424.786	1455.760	1467.787	1494.829
1554.723	1569.725	1659.867	1665.979	1704.003	1708.874
1918.034					

**22: 75-B4**

**Q9H0U4 (RAB1B\_HUMAN) Ras-related protein Rab-1B**

**[84 0.00095 6/12 34 %; 22.2K pI 5.55]**

990.493	1071.628	1087.541	1102.518	1119.542	1316.669
1348.664	1377.612	1457.729	1724.869	1798.800	2196.205

**27: 75-E4**

**P52565 (GDIR1\_HUMAN) Rho GDP-dissociation inhibitor 1**

**[77 0.0046 6/9 25 %; 23.2K pI 5.02]**

980.488	1245.553	1261.545	1470.745	1617.765	1751.834
1773.826	1798.795	1917.934			

**31: 75-B8 (Database: SwissProt 47.7; error tolerance 150 ppm)**

**P09104 (ENOG\_HUMAN) Gamma-enolase**

**[115 1.7e-07 11/21 30 %; 47.1K pI 4.91]**

963.472	991.525	1014.147	1018.774	1057.569	1088.601
1100.527	1130.622	1160.558	1235.656	1255.622	1406.728
1586.786	1599.780	1603.774	1615.754	1617.818	1631.757
1858.952	1911.364	1970.974			



**Plate 78 PMF**

Protein stain: Sypro Ruby

Database: SwissProt 47.8 (248831 sequences) – 05 Sep 2005

Taxonomy: Mammals (85577 sequences) or Human (54016 sequences)

Error tolerance: 100 ppm

Variable modifications: CAM-C, Met-ox, prop-C

Any deviations from these parameters are indicated adjacent to spot number.

**8: 78-C6 (Error tolerance: 20 ppm)****Q9NVA2 (SEP11\_HUMAN) Septin-1****[78 0.0014 11/40 26%; 49.2K pI 6.38]**

908.435	914.471	946.546	958.573	980.664	992.608
1002.632	1035.640	1077.576	1089.616	1123.709	1152.556
1211.766	1224.604	1233.672	1260.879	1286.651	1332.745
1361.771	1378.675	1405.817	1433.772	1439.780	1523.957
1537.865	1555.723	1581.937	1619.859	1641.860	1764.900
1922.902	1958.014	1962.948	1978.997	2003.064	2035.990
2070.944	2082.993	2150.129	2193.095		

**10: 78-B4 (Variable modifications: - prop-C)****P30740 (ILEU\_HUMAN) Leukocyte elastase inhibitor****[175 1.7e-13 16/26 37%; 42.7K pI 5.90]**

1003.574	1035.601	1079.650	1102.582	1149.571	1179.703
1194.531	1027.619	1218.596	1230.675	1240.588	1287.678
1305.689	1314.760	1327.670	1346.704	1427.810	1496.851
1510.734	1592.721	1602.764	1634.749	1650.791	1686.857
1785.903	2052.938				

**15: 78-A7 (Error tolerance: 110 ppm)****P00491 (PNPH\_HUMAN) Purine nucleoside phosphorylase****[82 0.00055 9/22 33%; 32.1K pI 6.45]**

968.512	1003.570	1022.578	1188.499	1204.494	1208.635
1224.664	1229.777	1231.762	1251.684	1417.790	1618.840
1654.795	1664.849	1741.730	1800.933	1816.947	1874.060
2009.026	2420.293	2434.280	2508.325		

**17: 78-C7 (Error tolerance: 40 ppm)****P62993 (GRB2\_HUMAN) Growth factor receptor-bound protein 2****[68 0.014 7/24 27%; 25.2K pI 5.89]**

904.363	918.481	928.480	932.454	962.520	968.503
986.552	1003.591	1091.534	1212.694	1251.682	1300.682
1427.861	1506.739	1512.693	1569.675	1618.846	1683.848
1715.914	1809.825	2003.002	2035.990	2361.098	2420.260

**19: 78-A3 (Error tolerance: 25 ppm)****P32119 (PRDX2\_HUMAN) Peroxiredoxin 2****[73 0.0045 7/28 32%; 21.7K pI 5.67]**

905.614	924.430	964.531	972.569	978.545	1021.582
1071.682	1098.570	1111.652	1133.626	1179.638	1194.653
1211.681	1233.668	1320.798	1388.833	1420.852	1563.734
1630.024	1683.830	1713.791	1734.975	1756.944	1863.072
1885.058	1944.144	2035.977	2072.258		

**23: 78-E7****O00194 (RB27B\_HUMAN) Ras-related protein Rab-27B****[82 0.00057 9/36 44%; 24.6K pI 5.35]**

942.488	964.487	1003.575	1009.595	1023.594	1029.582
1111.606	1132.524	1151.672	1168.630	1190.625	1212.702
1244.573	1261.697	1283.684	1361.840	1377.790	1427.854
1493.750	1533.772	1567.769	1584.770	1600.774	1616.772
1683.829	1743.912	1790.895	1809.834	1974.937	1996.935
2036.010	2084.017	2184.101	2367.130	2420.253	2495.246

**29: 78-H7****P67936 (PTM4\_HUMAN) Tropomyosin alpha 4 chain****[81 0.00071 9/27 27%; 28.4K pI 4.67]**

905.590	958.535	968.475	1003.574	1030.587	1148.554
1170.662	1205.646	1243.647	1272.636	1284.739	1298.762
1332.751	1379.824	1399.747	1446.731	1614.771	1742.859
1753.912	1923.938	1962.948	2003.036	2036.009	2420.267
2434.285	2508.312	2629.329			

## **Plate 88 PMF**

Protein stain: SYPRO Ruby

Database: SwissProt 48.6 (259907 sequences) – 16 Dec 2005

Taxonomy: unrestricted

Error tolerance: 50 ppm

Variable modifications: CAM-C, Met-Ox, Prop-C

Any deviations from these parameters are indicated adjacent to spot number.

### **4: 88-D1**

**P61157 (ARP3\_HUMAN) Actin-like protein 3**

**[76 0.006 7/13 18%; 47.3K 5.61]**

947.507	1028.545	1057.538	1128.587	1196.556	1214.576
1243.643	1297.666	1442.805	1448.778	1540.773	1610.840
1768.908					

### **5: 88-D2**

**P00.352 (AL1A1\_HUMAN) Retinal dehydrogenase 1**

**[95 7.8e-05 8/11 21%; 54.7K 6.29]**

939.287	1189.655	1205.659	1221.662	1434.772	1544.825
1589.813	1645.858	1700.811	1714.851	2005.938	

### **6: 88-F5**

**P50402 (EMD\_HUMAN) Emerin**

**[114 1e-06 9/18 40%; 29.0K 5.26]**

924.438	941.493	963.481	1064.555	1085.545	1203.665
1213.616	1250.576	1255.652	1340.653	1371.698	1379.652
1396.755	1593.781	1740.956	1922.797	1937.942	2160.985

### **20: 88-G4**

**Q15907 (RB11B\_HUMAN) Ras-related protein Rab-11B [77 0.0056 7/22 34%; 24.3K 5.65] and**

**P30085 (KCY\_HUMAN) UMP-CMP kinase [71 0.02 6/15 35%; 22.2K 5.44] and**

**P32119 (PRDX2\_HUMAN) Peroxiredoxin 2 [54 4/9 23%; 21.7K 5.67]**

924.461	944.527	964.572	1021.608	1080.611	1160.614
1179.682	1211.723	1248.637	1274.671	1289.781	1306.659
1392.708	1469.785	1479.806	1511.779	1520.787	1563.748
1585.921	1641.829	1863.090	2386.192		

### **25: 88-G5 (Error tolerance: 100 ppm)**

**O00299 (CLIC1\_HUMAN) Chloride intracellular channel protein 1**

**[93 0.00014 9/20 37%; 26.8K 5.09]**

957.523	963.503	987.542	1021.543	1037.583	1065.580
1078.545	1094.553	1255.660	1281.719	1328.690	1396.744
1458.794	1472.796	1480.745	1490.795	1572.831	1588.882
1601.858	1844.982				

### **30: 88-H6 (variable modifications: + prop-C)**

**P13489 (RINI\_HUMAN) Ribonuclease inhibitor**

**[60 0.28 6/10 17%; 49.8K 4.71]**

963.455	1209.675	1255.599	1323.594	1397.558	1513.739
1530.723	1612.756	1630.772	2202.889		

**Table 5: Proteins lost from the soluble fraction upon stimulation.**

Peptide mass lists are tabulated below, along with Mascot search summary for each protein spot.

MALDI plate and spot number

SwissProt accession number, SwissProt code, protein name

[MOWSE score, expect value, matching peptides/total peptides, percentage coverage; theoretical MW, pI]

Scores in red indicate a statistically significant identification ( $p < 0.05$ ).

**Plate 83**

Gel stain: SYPRO Ruby protein stain

Database: SwissProt 48.2 (259907 sequences) – 16 Dec 2005

Taxonomy: unrestricted

Error tolerance: 100 ppm

Variable modifications: CAM-C, Met-ox, Prop-C

**83-G1**

**P06733 (ENOA\_HUMAN) Alpha-enolase**

**[96 5.6e-05 7/8 22%; 47.0K 6.99]**

1143.692	1406.743	1425.769	1541.833	1556.839	1633.894
1713.916	1805.023				

**83-A3**

**P02794 (FRIH\_HUMAN) Ferritin heavy chain**

**[67 0.049 5/11 25%; 21.1K 5.30]**

1098.597	1135.662	1148.618	1153.656	1281.701	1294.700
1303.610	1345.634	1452.704	1643.805	1816.844	

**83-D4**

**P07339 (CATD\_HUMAN) Cathepsin D precursor**

**[105 1.7e-06 11/20 31%; 44.5K 6.10]**

963.466	1065.617	1160.670	1191.713	1196.723	1239.673
1255.678	1271.637	1312.710	1405.756	1421.768	1437.742
1462.678	1601.828	1617.804	1803.836	1858.909	1975.022
2004.992	2350.060				