

## Supporting Information

### **A simple, rapid and low-cost staining of phosphoprotein gel electrophoresis via fluorescent dye of purpurin**

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**Keywords:** Purpurin/Phosphoprotein stain/Pro-Q Diamond/SDS-PAGE

#### **Abbreviations**

MeOH, methanol/NaAc, Sodium acetate trihydrate.

## Supplementary Material

**Table S1.** List of the identified phosphopeptides from mice brains in 2-D SDS-PAGE with LC/MS.

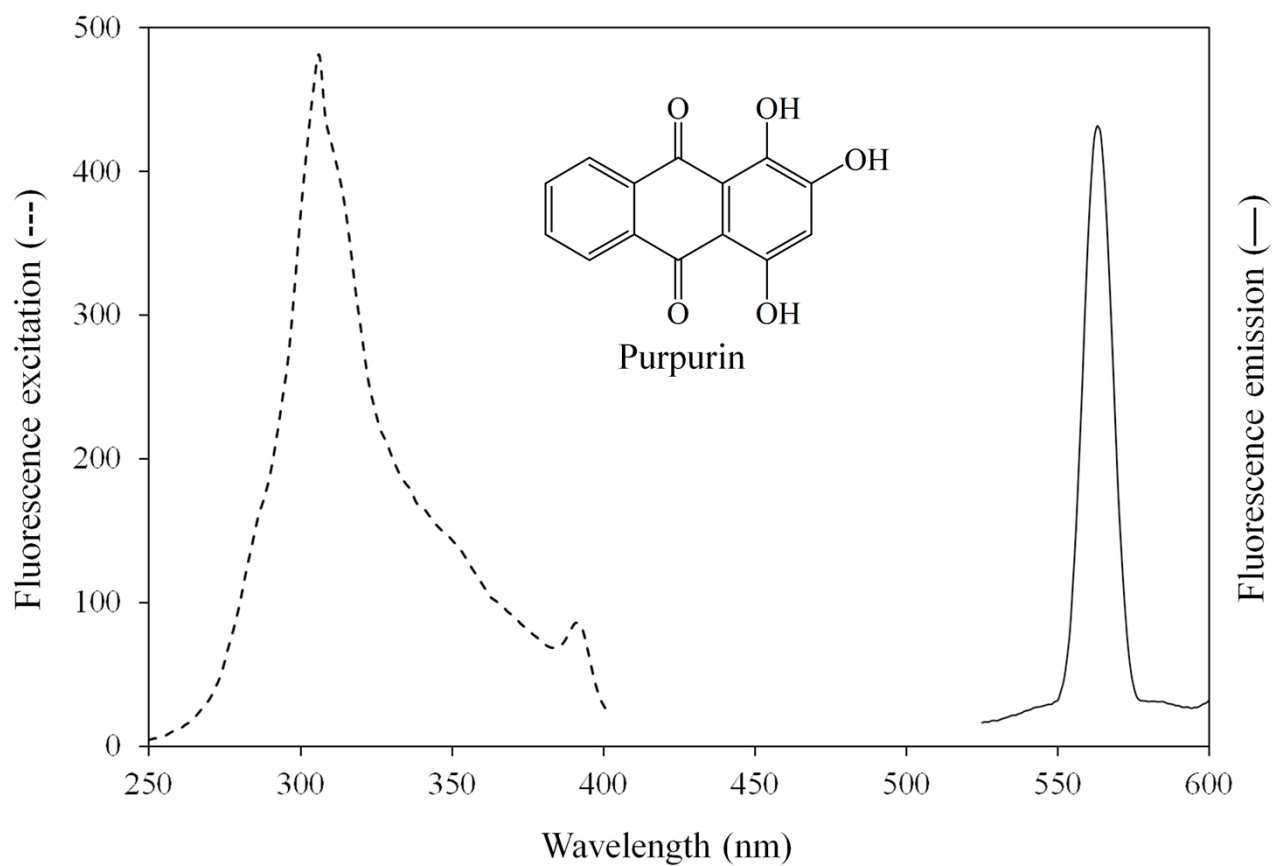
Spot No	Entry name	Description	Mr	pI	Phosphorylated Peptide	Ion Score	E-value	Ion Precursor	Ion Charge
1	NFM_MOUSE	Neurofilament medium polypeptide	95973	4.76	K.GVVTNGLDVS*PAEEK.K	59	5.6e-5	798.24	2
					K.AKSPMPKS*PVVEVKPKPEAK.A	51	0.00087	758.81	3
2	SPG7_MOUSE	Paraplegin	86170	4.96	R.VTS*GAQDDLRLK.V	46	0.011	635.93	2
3	NFL_MOUSE	Neurofilament light polypeptide	61528	4.62	K.KEES*AGEEQVAK.K	79	2.5e-6	693.00	2
					K.AEEAKDEPPS*EGEAEEEEK.E	75	5e-6	1092.17	2
4	VATB2_MOUSE	Ig gamma-3 chain C region	56857	4.57	R.S*GQVLEVS*GSK.A	46	0.012	626.35	2
5	NAB2_MOUSE	NGFI-A-binding protein 2	56827	6.40	K.IS*ET*AGT*RKGS*MS*NGHGSPGEK.A	65	9.8e-6	863.60	3
6	TBB3_MOUSE	Tubulin beta-3 cha	50842	4.82	K.VAVCDIPPRGLKMS*STFIGNSTAIQELFK.R	42	0.0034	1092.70	3
7	TBA4A_MOUSE	Tubulin alpha-4A chain	50697	5.37	K.LAEQCS*GLQGFLIYRSFSGGTGSGFTSLLMER.L	43	0.0091	1179.65	3
8	IGHG3_MOUSE	Ig gamma-3 chain C region	44472	6.68	R.AQTPQVY*T*IPPRE	39	0.0089	543.40	3
9	FGL1_MOUSE	Fibrinogen-like protein	36702	5.48	K.GSENS*FIDLGGK.R	42	0.009	652.52	2
10	LAMA2_MOUSE	Laminin subunit alpha-2	351638	5.75	R.CDCPPGYS*GLSCETCAPGFYR.L	55	0.0014	845.06	3
11	PSA3_MOUSE	Proteasome subunit alpha type-3	28615	5.29	K.ESLKEEDES*DDDNM.-	50	0.00013	876.17	2

**Table S2.** List of the identified phosphopeptides from human fibroblasts in 2-D SDS-PAGE with LC/MS.

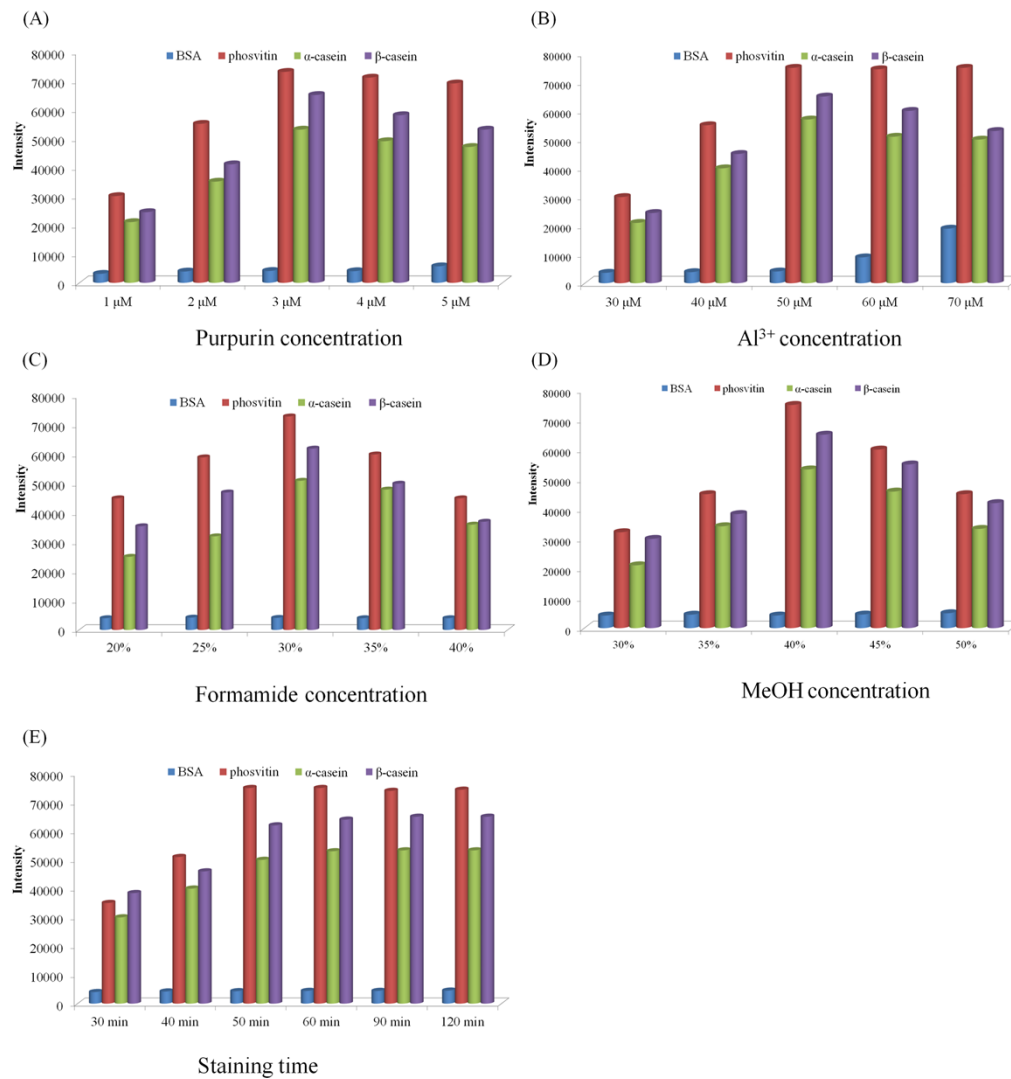
Spot No	Entry name	Description	Mr	pI	Phosphorylated Peptide	Ion Score	E-value	Ion Precursor	Ion Charge
1	DREB_HUMAN	Drebrin	71842	4.41	R.SPS*DSSTASTPVAEQIER.A	87	6.1e-8	971.79	2
					R.LREDENAEPVGTTYQKT*DAAVEMK.R	52	0.00016	925.83	3
2	FBP1L_HUMAN	Formin-binding protein 1-like	70390	6.12	K.MKDVY*EKNPQMGDPGS*LQPK.L	47	0.0027	818.68	3
3	FETUA_HUMAN	Alpha-2-HS-glycoprotein	40098	5.48	K.CDSSPDS*AEDVR.K	79	7.4e-7	709.60	2
					K.CDSSPDS*AEDVRK.V	69	0.00022	773.66	2
4	AKT3_HUMAN	RAC-gamma serine/threonine-protein kinase	56195	5.72	R.Y*FLLK.T	50	0.0033	763.46	3
5	PTN5_HUMAN	Formin-binding protein 1-like	70390	6.12	K.MKDVY*EKNPQMGDPGS*LQPK.L	51	0.00075	818.68	3
6	ARFG2_HUMAN	ADP-ribosylation factor GTPase-activating pro 2	57027	8.07	R.WDT*DAAWGMDR.V	47	0.0062	709.64	2
7	DC1L1_HUMAN	Cytoplasmic dynein 1 light intermediate chain 1	56829	6.01	R.T*PNRSVSSNVASVSPIPAGSK.K	60	0.00014	964.84	3
8	TPM1_HUMAN	Tropomyosin alpha-1 chain	32746	4.69	K.YS*EALKDAQEK.L	56	0.00094	681.21	2
9	CHKB_HUMAN	Choline/ethanolamine kinase	45585	5.35	R.EPVL*AAIATK.M	50	0.0047	589.66	2
10	ACTS_HUMAN	Actin, alpha skeletal muscle	42366	5.23	K.CDIDIRKDL*ANNVMS*GGTTMYPGIADR.M	46	0.0036	1108.47	3
11	CA198_HUMAN	Uncharacterized protein C1orf198	36438	5.60	R.MAS*MAAAIAASRS*AVMSGNRPLDDR.E	40	0.02	1363.33	2
12	CO029_HUMAN	Uncharacterized protein C15orf29	35201	5.18	K.IS*NFTNKNMK.E	41	0.0068	458.04	3

**Table S3.** Characteristics comparison of purpurin stain with Pro-Q Diamond stain for phosphoprotein detection in SDS-PAGE.

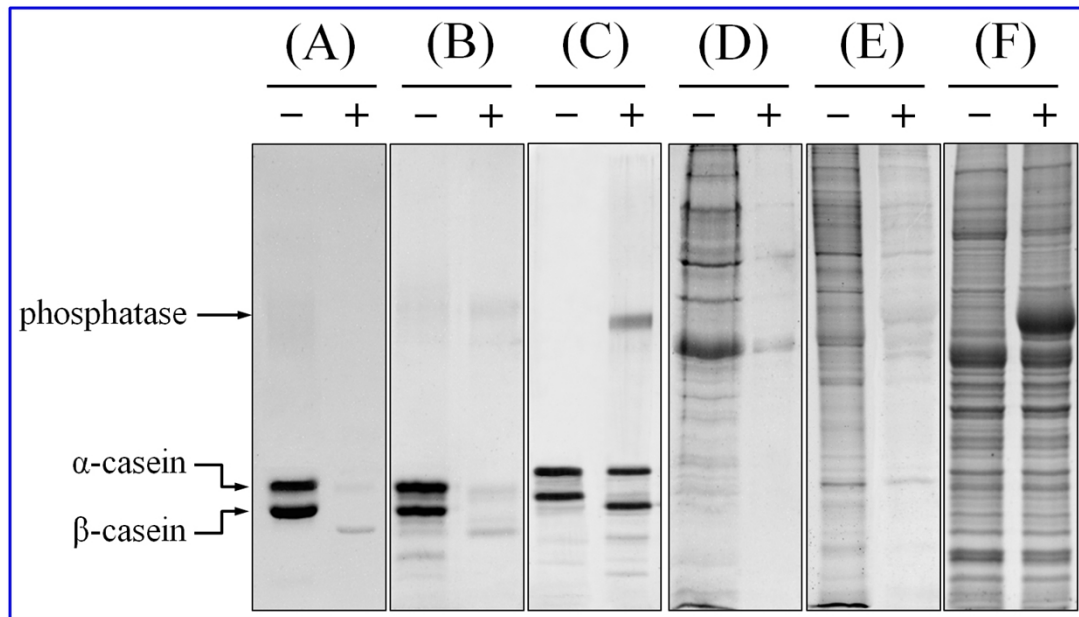
<b>Methods</b>	<b>Purpurin stain</b>	<b>Pro-Q Diamond stain</b>
<b>Elapsed time</b>	~60 min	~210 min
<b>Sensitivity</b>	4-8 ng	8-16 ng
<b>Cost (per use)</b>	0.8 \$	40 \$
<b>Steps</b>	2	9



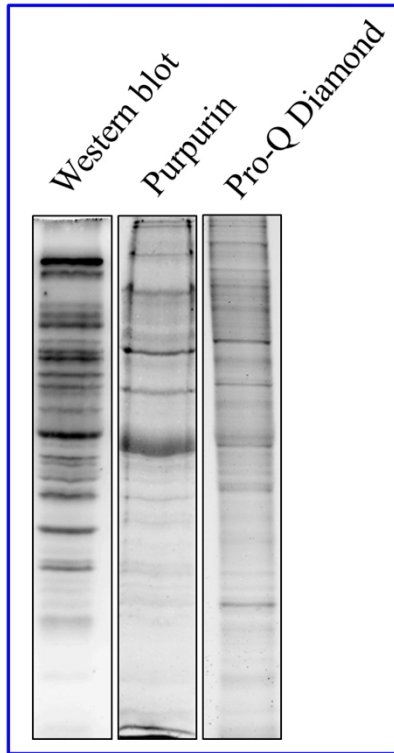
**Supplemental Figure 1.** Chemical structure and fluorescence excitation/emission spectra of purpurin.



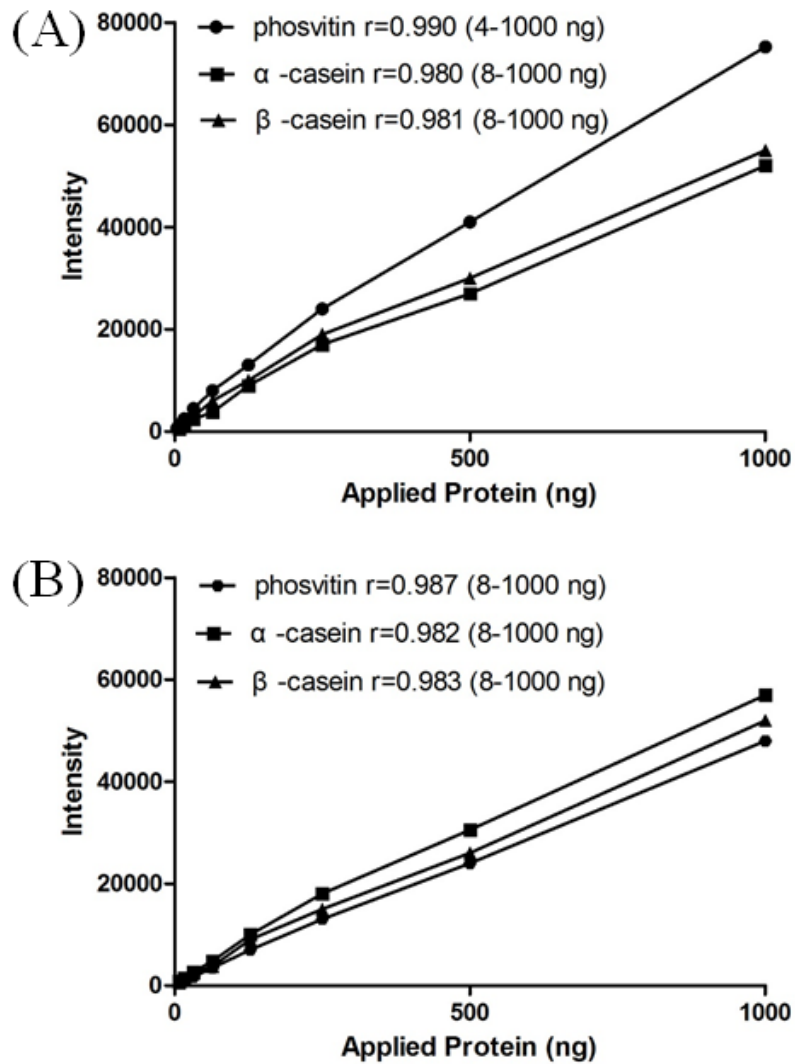
**Supplemental Figure 2.** Optimization of staining conditions. Phosphoprotein bands were quantified by using Image Lab 2010 software program. (A) Optimal purpurin concentration in staining solution. (B) Optimal Al<sup>3+</sup> concentration in staining solution. (C) Optimal formamide concentration in staining solution. (D) Optimal MeOH concentration in staining solution. (E) Optimal staining time.



**Supplemental Figure 3.** Specificity comparisons of purpurin stain with Pro-Q Diamond stain for the detection of non-treated phosphoproteins (-) and phosphatase-treated phosphoproteins (+). (A, B, C): marker protein  $\alpha$ -casein and  $\beta$ -casein, (D, E, F): mice brain total proteins. (A, D) purpurin stain, (B, E) Pro-Q Diamond stain; (C, F) SYPRO Ruby stain.

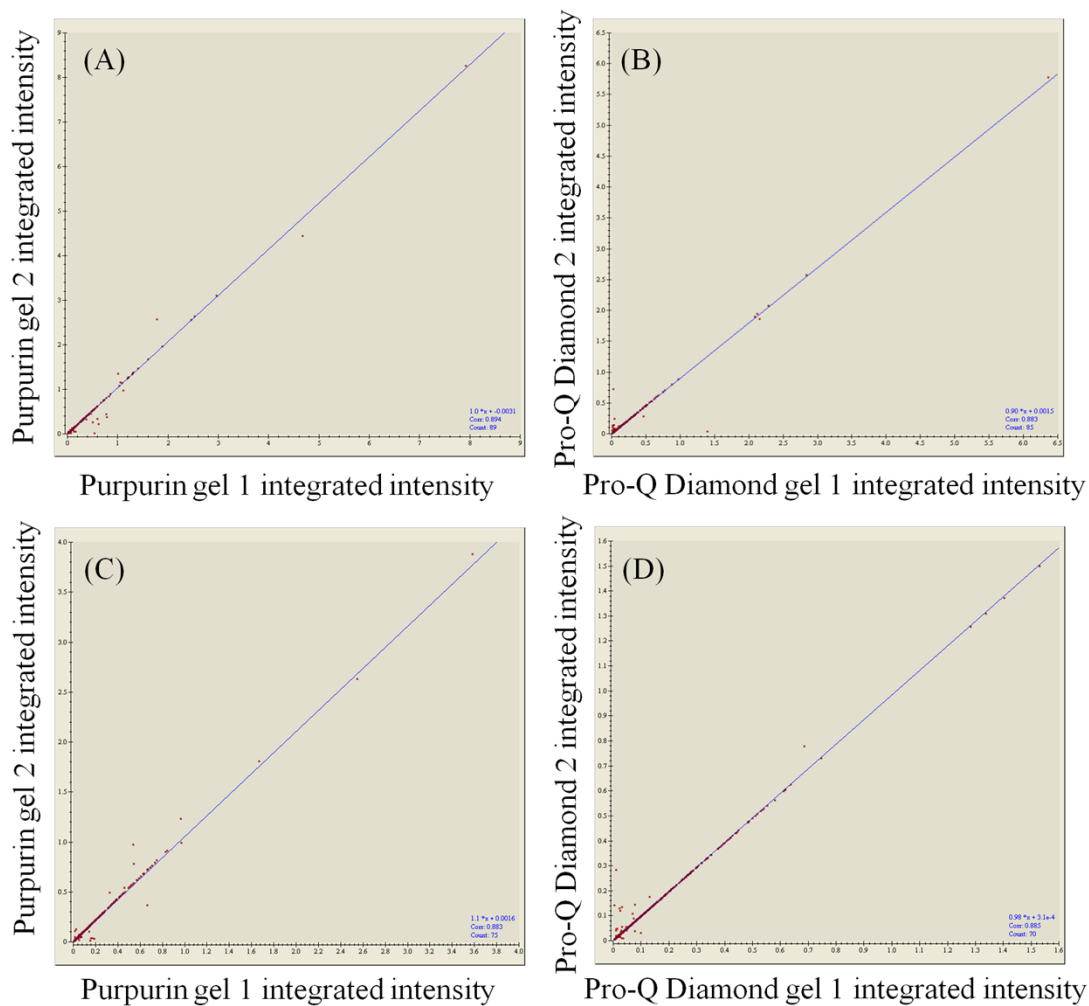


**Supplemental Figure 4.** Specificity comparisons of purpurin stain with western blot and Pro-Q Diamond stain for the detection of mice brain total proteins. For western blot, total proteins were separated by electrophoresis, transferred onto PVDF membrane and immunoblotted using anti-tyrosine antibody.



**Supplemental Figure 5.** Comparison of linear dynamic range between purpurin stain (A) and Pro-Q Diamond stain (B). Three representative phosphoproteins,  $\alpha$ -casein,  $\beta$ -casein and phosvitin were separated in 10% polyacrylamide gel. After staining, the protein bands were estimated by software of Image Lab 2010. The range of amount of proteins tested was 4-1000 ng.





**Supplemental Figure 6.** Quantitative correlation between corresponding matched spots in differently stained gels. (A, B) total proteins from mice brains; (C, D) total proteins from human fibroblasts. (A, C) Purpurin stain: three gels were stained with purpurin and matched with each other. The integrated intensities of matched spots from every gel were plotted against the corresponding matched spots from the other two gels. The average linear correlation coefficient of the line was determined to be 0.894 and 0.883, respectively. (B, D) Pro-Q Diamond stain: three gels were stained with Pro-Q Diamond and matched with each other. The average linear correlation coefficient of the line was determined to be 0.883 and 0.885, respectively.