

## Calculation of the Dye-to-Protein D/P-ratios

The **Dye-to-Protein Ratio** (D/P) or **Degree of Labeling** (DOL) can be calculated using the equation:

$$DP = \frac{A_{conj(\lambda_{max})} \mathcal{E}_p}{(A_{conj(278)} - xA_{conj(\lambda_{max})}) \mathcal{E}_{dye}}$$

where  $A_{conj(\lambda_{max})}$ ,  $A_{conj(278)}$  are the absorbencies of the dye—protein conjugate at the absorption maximum and at 278 nm respectively;

$\mathcal{E}_{dye}$  is the extinction coefficient of the dye at  $\lambda_{max}$ ,

$\mathcal{E}_p$  is the extinction coefficient of the protein at 278 nm.

For BSA:  $\mathcal{E}_p(278\text{ nm}) = 45,540\text{ M}^{-1}\text{cm}^{-1}$  and for IgG:  $\mathcal{E}_p(278\text{ nm}) = 201,700\text{ M}^{-1}\text{cm}^{-1}$ .

The factor  $x$  in the denominator accounts for dye absorption at 260 nm (for labeling of DNA) or at 278 nm (for labeling of proteins):

$$x = A_{dye(260/278)} / A_{dye(\lambda_{max})}$$

**x factor values:**

Dye	x	
	at 260 nm	at 278 nm
<b>K1-204</b>	11.3	8.15
<b>K4-205</b>	0.41	0.24
<b>K4-215</b>	1.05	0.78
<b>K4-225</b>	0.67	0.34
<b>K6-5052</b>	0.61	0.35
<b>K7-545</b>	6.80	3.30
<b>K7-547</b>	1.20	0.90
<b>K8-1341</b>	0.06	0.07
<b>K8-1342</b>	0.06	0.07
<b>K8-1352</b>	0.07	0.08
<b>K8-1357</b>	0.06	0.06
<b>K8-1367</b>	0.11	0.15
<b>K8-1384</b>	0.17	0.14
<b>K8-1388</b>	0.17	0.16
<b>K8-1407</b>	0.11	0.14
<b>K8-1626</b>	0.05	0.05
<b>K8-1641</b>	0.05	0.05
<b>K8-1642</b>	0.05	0.05
<b>K8-1649</b>	0.18	0.19
<b>K8-1663</b>	0.06	0.06
<b>K8-1667</b>	0.05	0.06
<b>K8-1671</b>	0.10	0.10
<b>K8-1672</b>	0.10	0.10
<b>K8-1682</b>	0.09	0.09
<b>K8-1902</b>	0.18	0.50
<b>K8-3002</b>	0.25	0.29

Dye	x	
	at 260 nm	at 278 nm
<b>K8-3335</b>	0.05	0.08
<b>K8-3345</b>	0.06	0.12
<b>K8-3346</b>	0.05	0.08
<b>K8-3402</b>	0.04	0.07
<b>K8-3403</b>	0.04	0.07
<b>K8-5035</b>	0.05	0.05
<b>K8-5036</b>	0.05	0.05
<b>K8-5045</b>	0.06	0.08
<b>K8-5046</b>	0.05	0.05
<b>K8-5075</b>	0.09	0.10
<b>K8-5402</b>	0.04	0.05
<b>K8-5403</b>	0.04	0.05
<b>K8-7045</b>	0.06	0.08
<b>K8-7046</b>	0.05	0.06
<b>K8-7049</b>	0.19	0.17
<b>K8-7055</b>	0.11	0.10
<b>K8-7522</b>	0.06	0.06
<b>K9-3152</b>	0.29	0.26
<b>K9-4119</b>	0.67	0.16
<b>K9-4142</b>	0.16	0.12
<b>K9-4148</b>	0.15	0.12
<b>K9-4149</b>	0.15	0.12
<b>K9-4154</b>	0.23	0.23
<b>K9-4159</b>	0.25	0.22
<b>K9-4169</b>	0.05	0.03
<b>K9-4179</b>	0.14	0.10