SETA BioMedicals
Fluorescent Tools for BioMedical Applications

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Technical Note
Calculation of the Dye-to-Protein Ratio

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

$$DP = \frac{A_{conj(\lambda max)}\varepsilon_p}{(A_{conj(278)} - xA_{conj(\lambda max)})\varepsilon_{dye}}$$

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;

ε_{dye} is the extinction coefficient of the dye at λ_{max},

ε_p is the extinction coefficient of the protein at 278 nm.

For BSA: ε_p (278 nm) = 45,540 M^{-1}cm^{-1} and for IgG: ε_p (278 nm) = 201,700 M^{-1}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):

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

Recommended *x* factor values:

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

Dye	x	
	at 260 nm	at 278 nm
K8-1671	0.10	0.10
K8-1672	0.10	0.10
K8-1682	0.09	0.09
K8-1902	0.18	0.50
K8-3002	0.25	0.29
K8-3335	0.05	0.08
K8-3402	0.04	0.07
K8-3403	0.04	0.07
K8-5035	0.05	0.05
K8-5036	0.05	0.05
K8-5075	0.09	0.10
K8-5402	0.04	0.05
K8-5403	0.04	0.05
K8-7049	0.19	0.17
K8-7055	0.11	0.10
K9-4119	0.67	0.16
K9-4142	0.16	0.12
K9-4148	0.15	0.12
K9-4149	0.15	0.12
K9-4169	0.05	0.03
K9-4179	0.14	0.10