

## Quantification of Eluted Dye

### Material required

**\*Comment \*All procedures with dye-eluting solution to be performed in chemical hood**

- Pyridine                                       Acetic Acid  
 Coomassie Brilliant Blue R-250       Methanol

### fixing solution

Final	Name	Stock sol.	AMT (50 ml)	AMT (200 ml)
7.5%	Acetic Acid	100%	<input type="checkbox"/> 3.75 ml	<input type="checkbox"/> 15 ml
	ddH <sub>2</sub> O		<input type="checkbox"/> 46 ml	<input type="checkbox"/> 185 ml

### Staining solution

Final	Name	Stock sol.	AMT (50 ml)	AMT (200 ml)
0.5%	Coomassie Brilliant Blue R-250		<input type="checkbox"/> 0.25g	<input type="checkbox"/> 1g
7.5%	Acetic Acid	100%	<input type="checkbox"/> 3.75 ml	<input type="checkbox"/> 15 ml
50%	Methanol	100%	<input type="checkbox"/> 25 ml	<input type="checkbox"/> 100 ml
	ddH <sub>2</sub> O		<input type="checkbox"/> 21 ml	<input type="checkbox"/> 85 ml

### De-staining solution 1

Final	Name	Stock sol.	AMT (50 ml)	AMT (200 ml)
7.5%	Acetic Acid	100%	<input type="checkbox"/> 3.75 ml	<input type="checkbox"/> 15 ml
50%	Methanol	100%	<input type="checkbox"/> 25 ml	<input type="checkbox"/> 100 ml
	ddH <sub>2</sub> O		<input type="checkbox"/> 21 ml	<input type="checkbox"/> 85 ml

### De-staining solution 2

Final	Name	Stock sol.	AMT (50 ml)	AMT (200 ml)
7.5%	Acetic Acid	100%	<input type="checkbox"/> 3.75 ml	<input type="checkbox"/> 15 ml
5%	Methanol	100%	<input type="checkbox"/> 2.5 ml	<input type="checkbox"/> 10 ml
	ddH <sub>2</sub> O		<input type="checkbox"/> 43.75 ml	<input type="checkbox"/> 175 ml

### Dye eluting solution

Final	Name	Stock sol.	AMT (50 ml)	AMT (200 ml)
25%	Pyridine	100%	<input type="checkbox"/> 12.5 ml	<input type="checkbox"/> 50 ml
	ddH <sub>2</sub> O		<input type="checkbox"/> 47.5 ml	<input type="checkbox"/> 150 ml

## Procedure

Soak the gel in **Fixing sol.** for **30 min**

Soak the gel in **Staining sol.** for **12-16 hrs**

Soak the gel in **De-staining sol. 1**, changing solution several times in **2hrs**  
Soak the gel in **De-staining sol. 2**, changing solution **2~3 times** until no **background**

Cut bands and put into **Dye eluting sol.**  
Extract mixture shaken **overnight** at **RT**

Measuring Absorption by **605nm**

## References

Fenner C, Traut RR, Mason DT, Wikman-Coffelt J (1975) Quantification of Coomassie Blue stained proteins in polyacrylamide gels based on analyses of eluted dye. *Anal Biochem* 63(2): 595-602