

Preparation of DNP-KLH, HGG

- Materials:
- KLH (m.w. 10^5) 50 mg = 5×10^{-7} moles
 - DNFB, 8M in ~~Dioxane~~ (immediately before use, dilute 1:8 to yield 1 molar in Dioxane)
 - HGG (1.5×10^5 m.w.)
 - PBS
 - .5M NaHCO_3

KLH Procedure:

1. To 50 mg KLH (5×10^{-7} moles) in 5 cc of .5M NaHCO_3 (pH > 8) stirring slowly at room temp, add 20 μ (20×10^{-6} moles) 1M DNFB in Dioxane. This yields

$$\frac{20 \text{ moles DNFB}}{1 \text{ mole KLH}}$$

IN DARK

2. Stir at room temp for 1-3 hours.
3. Dialyze against 4 liters PBS for at least 48 hours or until dialysate is clear.
4. Centrifuge at 10,000 rpm in Sorvall and take supernatant for O.D.'s.
5. Calculate yield using following information:

$$\text{OD}_{280} \text{ KLH} = 1.55 \quad E_{\text{ICM}}^{0.1\%}$$

$$\text{OD}_{360} \text{ DNP} = 1.74 \times 10^4 \quad E_{\text{ICM}}^M$$

$$\text{OD}_{280} \text{ DNP} = 0.385 \times 1.74 \times 10^4 \times \frac{1}{\text{hole}} E_{\text{ICM}}^M = 0.64 \times 10^4 \quad (\text{e.g., if } \text{OD}_{360} = 1, \text{OD}_{280} = 0.385)$$

Therefore:

$$\text{OD}_{280} \text{ due to protein} = \text{OD}_{280} - (\text{OD}_{360} \times 0.385)$$

$$\text{OD}_{280} \text{ protein} / 1.55 = \text{mg/ml KLH}$$

$$\text{OD}_{360} / 1.74 \times 10^4 = \text{molarity of DNP}$$

$$\text{Molarity of KLH} = \text{mg/ml KLH} / 10^5$$

$$\text{Molarity of DNP} / \text{molarity KLH} = \text{Molar ratio. above procedure should give } \sim 10 \text{ DNP/KLH (} 10^5 \text{ m.w.)}$$

mg/ml BGG / 1.5×10^5

HGG Procedure: same as above, but substitute HGG $\text{OD}_{280} = 1.4 E_{\text{ICM}}^{.1\%}$

Procedure should give ~ 10 DNP/HGG

Note: Have been using 100 μ DNP₁₀-KLH alum ppt. in 0.2m I.P. for priming mice.

Note: This is too high for BGG reduce by factor of 3 See: Handbook of Imm. Weir Page 29.3

*2.9
3.07
1.29
1.78*

