

## Dot Blot/ Attempted visualization with anti-HIS antibodies

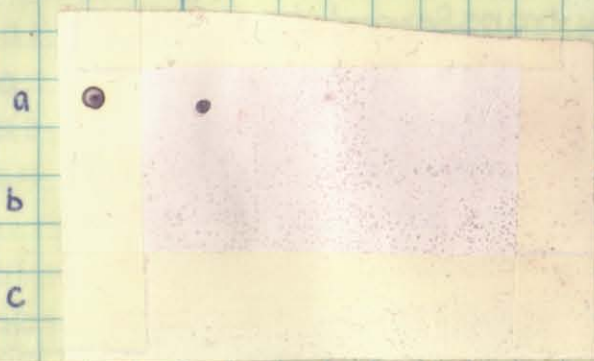
Ac-HHHHHHGKW-NH<sub>2</sub> : 15  $\mu$ g (11,970 nmol) dissolved in 20  $\mu$ L H<sub>2</sub>O  
 concentration : 750  $\mu$ g/ $\mu$ L ( $\approx$  600 nmol/ $\mu$ L)

Ac-HHHHGKW-NH<sub>2</sub> : 1.8 mg (1,184 nmol) dissolved in 10  $\mu$ L H<sub>2</sub>O  
 concentration : 180  $\mu$ g/ $\mu$ L (184 nmol/ $\mu$ L)

|    |           |  |                         |
|----|-----------|--|-------------------------|
| a1 | 3 $\mu$ L | of a 0.01 nmol/ $\mu$ L soln. (2.3 $\mu$ g, 0.03 nmol)     | } His6-Pax-FLAG protein |
| a2 | 1 $\mu$ L | of a 0.01 nmol/ $\mu$ L soln. (0.75 $\mu$ g, 0.01 nmol)    |                         |
| a3 | 1 $\mu$ L | of a 0.001 nmol/ $\mu$ L soln. (0.075 $\mu$ g, 0.001 nmol) |                         |
| a4 | 1 $\mu$ L | of a 0.0001 nmol/ $\mu$ L soln. (7.5 ng, 0.0001 nmol)      |                         |

|    |           |   |                                |
|----|-----------|---|--------------------------------|
| b1 | 3 $\mu$ L | of a 600 nmol/ $\mu$ L soln. (2.3 $\mu$ g, 1.8 $\mu$ mol) | } Ac-HHHHHHGKW-NH <sub>2</sub> |
| b2 | 1 $\mu$ L | of a 600 nmol/ $\mu$ L soln. (750 ng, 600 nmol)           |                                |
| b3 | 1 $\mu$ L | of a 60 nmol/ $\mu$ L soln. (75 ng, 60 nmol)              |                                |
| b4 | 1 $\mu$ L | of a 6 nmol/ $\mu$ L soln. (7.5 ng, 6.0 nmol)             |                                |

|    |           |   |                              |
|----|-----------|---|------------------------------|
| c1 | -         |   | } Ac-HHHHGKW-NH <sub>2</sub> |
| c2 | 1 $\mu$ L | of a 180 nmol/ $\mu$ L soln. (180 nmol, 180 ng) |                              |
| c3 | 1 $\mu$ L | of a 18 nmol/ $\mu$ L soln. (18 ng, 18 nmol)    |                              |
| c4 | 1 $\mu$ L | of a 1.8 nmol/ $\mu$ L soln. (1.8 ng, 1.8 nmol) |                              |



1 2 3 4  
 mouse anti-hexahistidine



1 2 3 4  
 mouse anti-tetrahistidine 1° antibody  
 3  $\mu$ g in 30 mL (Quiagen selector kit)