

# H3K36me3\_Cell Signaling\_4909\_2\_DB\_PchAb 1380

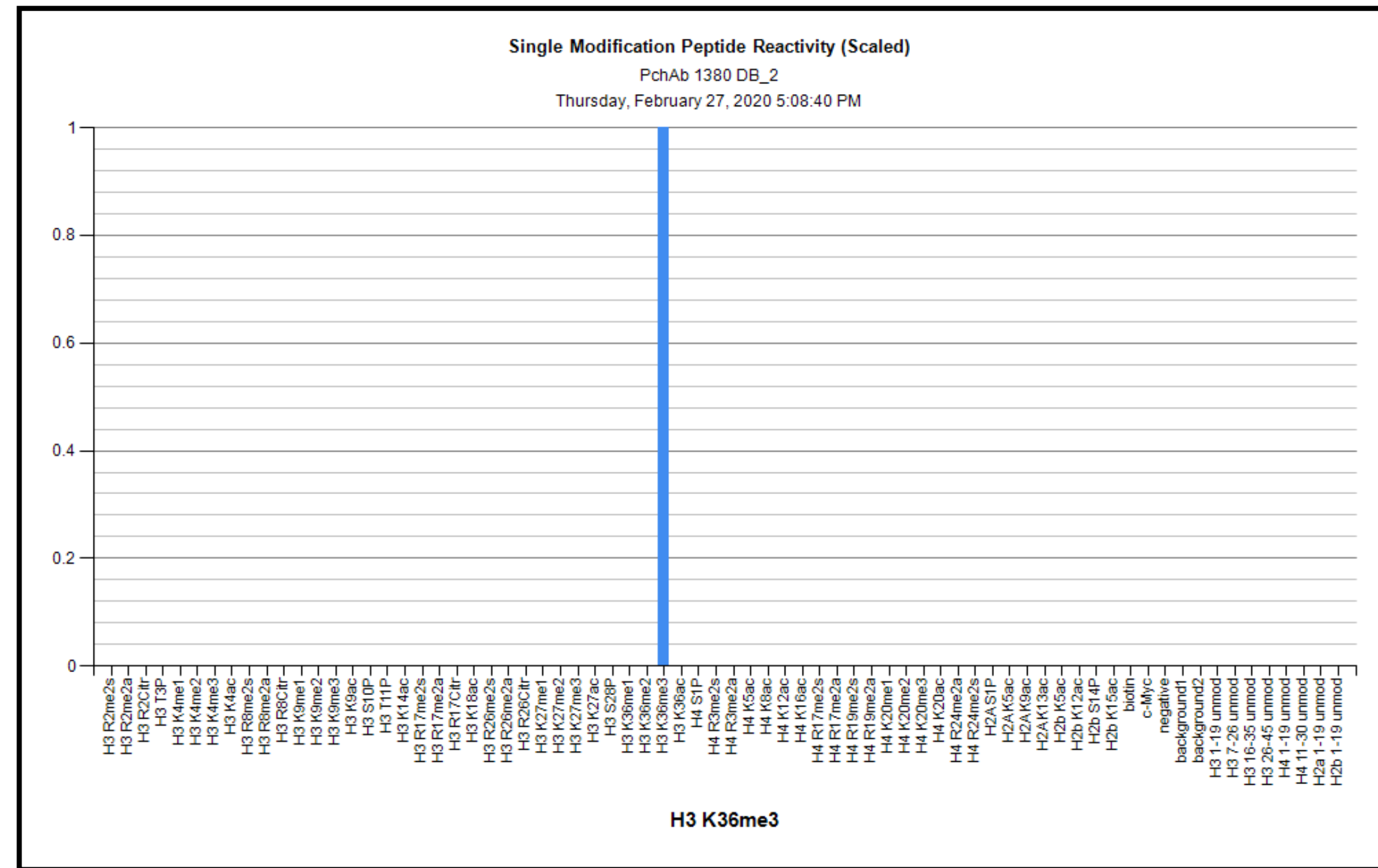
Test Date: 2-27-2020  
User: RI

**Primary antibody:**  
Antibody: H3K36me3  
Vendor: Cell Signaling  
Part number: 4909  
Lot number: 2  
Dilution: 1:10000  
Incubation: O/N at 4C

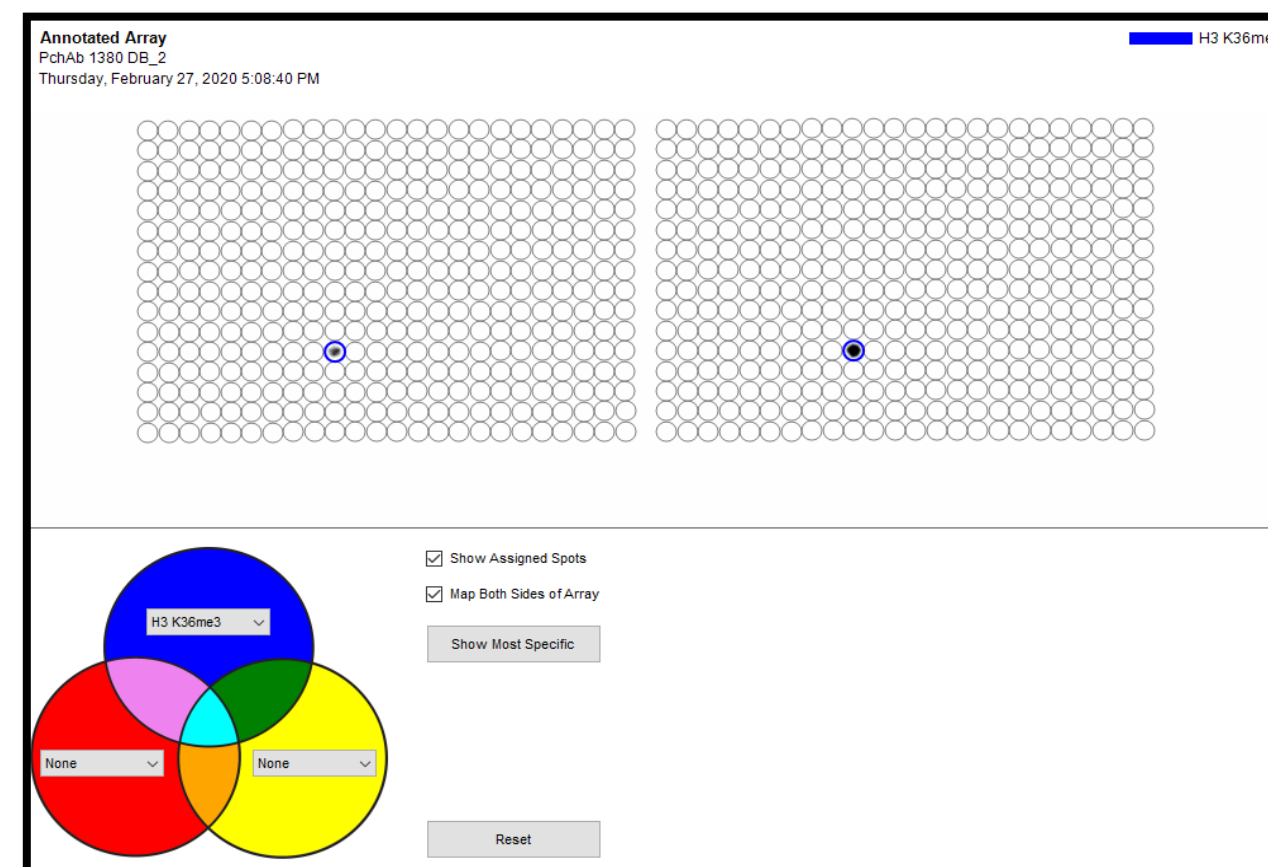
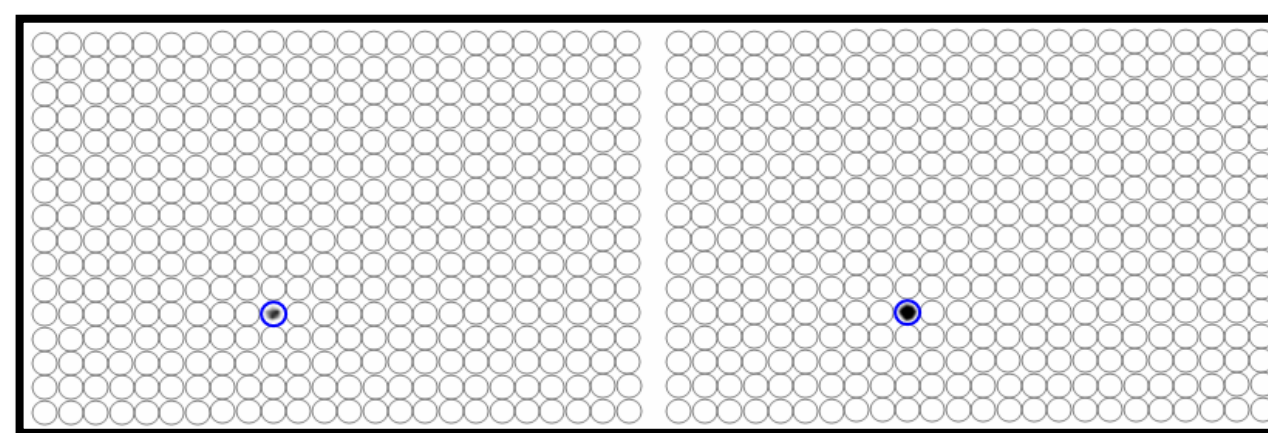
**Secondary antibody:**  
Vendor: Abcam  
Part number: ab6721  
Lot number: GR119224-3  
Dilution: 1:2500  
Incubation: 1 hr@ RT

- Protocol:**
- immerse array in 4 mL of blocking solution containing (TBST containing 5% non-fat dried milk)
  - incubate on a rotator for 4 hours at room temperature
  - carefully pour off buffer
  - perform a quick rinse (30 seconds) with TBST buffer
  - wash 3 x 5 minutes in TBST
  - dilute primary antibody in 4 mL of Blocking solution (TBST w 5% BSA)
  - incubate on a rotator overnight at 4°C
  - carefully pour off buffer
  - perform a quick rinse (30 seconds) with TBST buffer
  - wash 3 x 5 minutes in TBST
  - dilute secondary antibody in 4 mL of Blocking solution
  - incubate on a rotator for 1 hour at room temperature
  - carefully pour off buffer
  - perform a quick rinse (30 seconds) with TBST buffer
  - wash 3 x 5 minutes in TBST
  - detect using Chemi-Doc XR

Reactivity of test antibody with control peptides and singly modified peptides  
Graph is scaled so that the intended primary reactivity is set to 1



Gridded image of the array



Cross inhibition of reactivity by other modifications of the same peptide  
Graph is scaled so that saturation of signal = 1

There is no graph for H3K36me3 since there is only one spot on the array that contains this, namely, the singly modified peptide