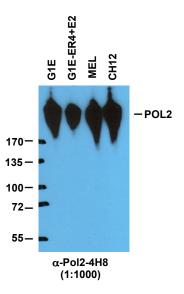
## **ENCODE DCC Antibody Validation Document**

Date of Sub	mission 9-14-12				
Name: Che	Name: Cheryl Keller		Email: cak142@psu.edu		
	Lab	Hardison			
Antibody Na	ame: RNA polymerase II C	CTD repeat	Target: POL2		
	Compa Source	, hoodin			
Catalog Numb	per, database ID, laboratory	ab5408	Lot Number	722997, 962594	
Antibody Description: Mouse monoclonal [4H8] to RNA polymerase II CTD repeat YSPTSPS synthetic peptide (human); sequence repeated multiple times in C-terminal domain of RNA pol II largest subunit; recognizes both unphosphorylated and phosphorylated forms					
Target I Description:	Polymerase (RNA) II (DNA Entrez Gene: 5430	A directed) polypept	ide A, 220kDa; ~217k	Da	
Spe	ecies Target Mouse		Species Host Mouse		
Val	idation Method #1 Wester	n Blot	Validation Method #2		
Purification Method			Polyclonal/ Monoclonal		
Vendor URL: http://www.abcam.com/RNA-polymerase- II-CTD-repeat-YSPTSPS-antibody-4H8-					
Reference (Pl/ Publication Information)					
Please complete the following for antibodies to histone modifications: if your specifications are not listed in the drop-down box, please write-in the appropriate information					
Histone Name	AA modified		AA Position	Modification	n

## Hardison Lab @ Penn State University Pol2-4H8 antibody validation



Pol2-4H8 antibody: Abcam POL24H8, Cat# ab5408

## Validation of Pol2-4H8 antibody.

## Western blot analysis of POL2 expression in various mouse cell lines.

Whole cell extracts prepared from G1E, G1E-ER4+E2, MEL, and CH12 cells were separated by SDS-PAGE and transferred to PVDF using standard immunoblotting methods. POL2 expression was detected using mouse anti-POL24H81 (ab5408, 1:1000) followed by incubation with anti-mouse secondary antibody (1:5000) and detected by ECL Plus (Amersham Biosciences). As expected, a single band of approximately 260 kDa was detected (predicted molecular weight: 217 kDa) in all cell lines tested.