

ENCODE DCC ANTIBODY VALIDATION DOCUMENT

Date of Submission: 6/22/11

Name: Zarmik Moqtaderi

Email: Zarmik@hms.harvard.edu

Lab: Struhl

Antibody Name: BRF2

Target: BRF2

Company/Source: Donated by Dr. RJ White, The Beatson Institute for Cancer Research antibody number 4295

Catalog number or laboratory database ID: antibody number 4295

Lot Number:

Antibody Description: Antibody 4295 against Brf2 was raised by immunizing rabbits with keyhole limpet hemocyanin coupled to synthetic peptides VSRSQQRGLRRVRDLC and SDSEIEQYLRTPQEVR, corresponding to human Brf2 residues 66-80 and 385-400, respectively

Target Description: Brf2 is a component of an alternate form of the RNA Polymerase III transcription factor TFIIB.

Target Species: Human

Host Species: Rabbit

Validation Method 1: Western Blot

Validation Method 2: Correlation with known Pol III factors at expected loci.

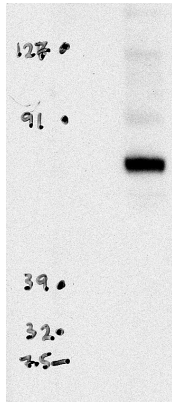
Purification Method:

Polyclonal/Monoclonal: Polyclonal

Reference: Moqtaderi, Z., Wang, J., Raha, D., White R.J., Snyder M., Weng Z., and Struhl, K.

Genomic binding profiles of functionally distinct RNA polymerase III transcription complexes in human cells. *Nat. Struct. Mol. Biol.* 2010 May;17(5):635-40. Epub 2010 Apr 25.

Validation 1: Western blotting with this antibody shows a strong band at the appropriate size in the ENCODE cell line GM12878:



Validation 2: Brf2 is a component of an alternative form of the Pol III transcription factor TFIIB, which is involved in the transcription of a small minority group of Pol III-transcribed genes with upstream extragenic promoters. Brf2 is associated with the expected loci. Although Brf2-bound loci recruit high levels of the Pol III subunit Rpc155, these loci are not associated with the more common TFIIB subunit Brf1. This is exactly the expected result for a component of an alternative TFIIB subunit.

This is illustrated in Figure 3 from:

Moqtaderi, Z., Wang, J., Raha, D., White R.J., Snyder M., Weng Z., and Struhl, K. Genomic binding profiles of functionally distinct RNA polymerase III transcription complexes in human cells. *Nat. Struct. Mol. Biol.* 2010 May;17(5):635-40. Epub 2010 Apr 25.

