## **ENCODE DCC Antibody Validation Document**

| Date of Submission   |
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|  |
|  |
| Name: Email:   |
|  |
| Lab  |
| Lab  |
|  |
| Antibody Name: Target:   |
|  |
| Company/   |
| Source:  |
|  |
| Catalog Number, database ID, laboratory  Lot Number                  |
|  |
|  |
| Antibody   |
| Description:   |
|  |
|  |
| Target   |
| Target Description:  |
|  |
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| Species Toyant Species Hest  |
| Species Target Species Host  |
|  |
| Validation Method #1 Validation Method #2                            |
|  |
| Purification Polyclonal/   |
| Method Monoclonal  |
| Vicilia IIDI   |
| Vendor URL:  |
| eference (PI/  |
| ublication   |
| formation)   |
| ease complete the following for antibodies to histone modifications: |
| your specifications are not listed in the drop-down box,             |
| ease write-in the appropriate information                            |
| istone Name AA modified AA Position Modification                     |
| istone ivalile   AA modified   AA Fosition   Iviodification          |

| Validation #1<br>Analysis            |  |  |  |  |  |
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| Insert Validation Image (click here) |  |  |  |  |  |



Lane 1 Input lysate

Lane 2 Bound material from IP

Lane 3 Bound material from IP

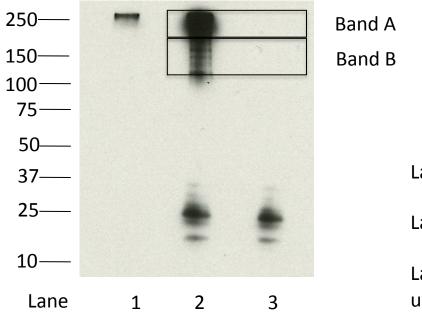
using non-specific IgG

Western Blot analysis of immunoprecipitated proteins from K562 nuclear extracts detected using anti PollIS2 antibody (ab5095). Expected protein band 240 kD.

| Validation #2<br>Analysis            |  |  |  |  |  |  |
|--------------------------------------|--|--|--|--|--|--|
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| Insert Validation Image (Click here) |  |  |  |  |  |  |

## Immunoprecipitation assay (IP) + mass spectrometry assay

MW pollIS2 (ab5095) (R) 240 kD



Lane 1 Input lysate

Lane 2 Bound material from IP

Lane 3 Bound material from IP using non-specific IgG

| Spectra        | Name of Protein  | Count of Peptides  | Ratio (pollIS2/IgG<br>Control) |
|----------------|--|--------------------|--------------------------------|
| эреспа         | Name of Frotein  | count of 1 eptides | Controly                       |
| pollIS2_Band A | DNA-directed RNA polymerase II                                       | 98                 | NOT IN CONTROL IP              |
| pollIS2_Band A | Isoform 1 of Nucleolar RNA helicase 2                                | 10                 | 0.666666667                    |
| pollIS2_Band A | Stromal interaction molecule 1                                       | 10                 | NOT IN CONTROL IP              |
| pollIS2_Band A | Heat shock protein HSP 90-beta                                       | 5                  | 0.454545455                    |
| pollIS2_Band A | Putative uncharacterized protein RECQL5                              | 4                  | NOT IN CONTROL IP              |
| pollIS2_Band A | Tubulin beta-1 chain   | 1                  | NOT IN CONTROL IP              |
| pollIS2_Band B | DNA-directed RNA polymerase II                                       | 23                 | NOT IN CONTROL IP              |
| pollIS2_Band B | Stress-70 protein, mitochondrial                                     | 10                 | 0.357142857                    |
| pollIS2_Band B | Isoform 2 of Heat shock cognate 71 kDa protein                       | 5                  | NOT IN CONTROL IP              |
| pollIS2_Band B | Nucleolar protein 5A (Fragment)                                      | 4                  | 0.5                            |
| pollIS2_Band B | X-ray repair complementing defective repair in Chinese hamster cells | 4                  | 0.44444444                     |
| polIIS2_Band B | insulin-like growth factor 2 mRNA-binding protein 1 isoform 2        | 3                  | NOT IN CONTROL IP              |