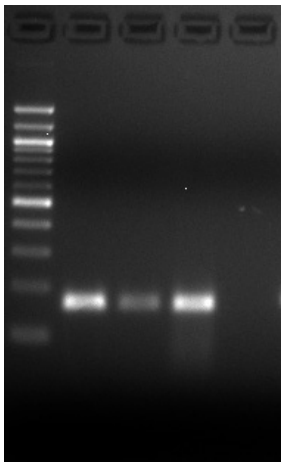
ChIP-IT® Control Kit – Human

Catalog No: 53010

Format: 5 rxns

Quality Control: ChIP-IT® Control Kit – Human is quality control tested in combination with Active Motif's ChIP-IT® Express Kit (Catalog No. 53008).

HeLa cells were grown, fixed and used to prepare chromatin as described in the ChIP-IT Express manual. ChIP reactions were then performed using 2 µg RNA pol II antibody plus 2 µg bridging antibody or 2 µg negative control IgG. The immunoprecipitated DNA and the control Input DNA were then used in endpoint PCR using the GAPDH control primers (Figure 1). The reactions were cycled for 36 repetitions. The positive control GAPDH primers generate a 166 bp product which should be enriched in the RNA pol II and Input samples. Signal in the Negative IgG samples represents non-specific background.



Lane Template Primers
1 DNA Ladder --
2 RNA pol II GAPDH
3 Negative IgG GAPDH
4 Input DNA GAPDH
5 H_2O control GAPDH

Kit Components:

50 µl RNA pol II mouse monoclonal antibody (0.2 µg/µl) (also sold as Cat. No. 39097)
50 µl Bridging antibody (1 µg/µl) (also sold as Cat. No. 53017)
50 µl Negative control mouse IgG (0.2 µg/µl)
400 µl GAPDH primer mix (2.5 µM)
1.5 ml 10X PCR buffer
1.5 ml 10X PCR loading dye.

Endpoint PCR Analysis

We recommend the following PCR conditions:
9.8 µl DEPC H_2O
2.5 µl 10X PCR Buffer
2.5 µl 10X PCR Loading Dye
1.0 µl dNTPs (5 mM mix)
0.2 µl Taq polymerase
4.0 µl GAPDH control primer mix
5.0 µl ChIP DNA
25 µl Total Volume

Reactions were cycled 36 times with the following steps per cycle:
94°C denaturing for 20 seconds
59°C annealing for 30 seconds
72°C extension for 30 seconds

Storage and Guarantee: The ChIP-IT Control Kit – Human components are shipped on dry ice. The negative control IgG antibody should be stored at 4°C, all other components can be stored at -20°C.

This product is guaranteed for 6 months from date of receipt under the correct storage conditions. Aliquot the antibodies to avoid exposing to multiple freeze-thaw cycles.