

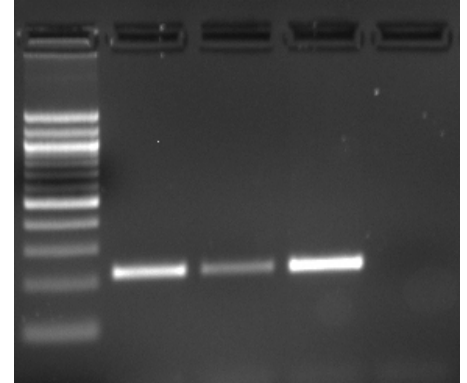
## ChIP-IT® Control Kit – Rat

**Catalog No: 53012**

**Format:** 5 rxns

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

Rat Nb2 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 Beta-actin control primers (Figure 1). The reactions were cycled for 30 repetitions. The positive control primers generate a 223 bp product which should be enriched in the RNA pol II and Input samples. Signal in the Negative IgG samples represents non-specific background.



### 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 Beta-actin primer mix (2.5 µM)  
 1.5 ml 10X PCR buffer  
 1.5 ml 10X PCR loading dye

Lane Template Primers  
 1 DNA Ladder --  
 2 RNA pol II Beta-actin  
 3 Negative IgG Beta-actin  
 4 Input DNA Beta-actin  
 5 H<sub>2</sub>O control Beta-actin

### Endpoint PCR Analysis

We recommend the following PCR conditions:

9.8 µl DEPC H<sub>2</sub>O  
 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 Beta-actin primer mix  
 5.0 µl ChIP DNA

### 25 µl Total Volume

Reactions were cycled 30 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 – Rat 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.