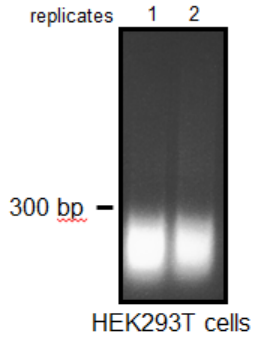
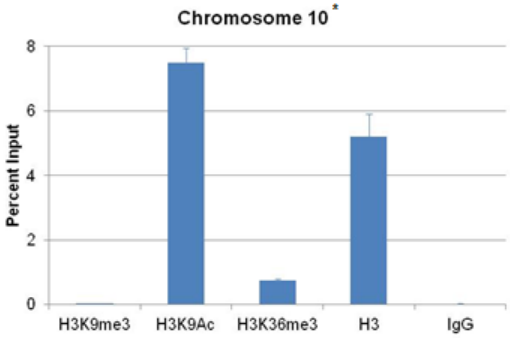
Mammalian Chromatin Prep & ChIP

Example protocols and results are based on customer feedback.





*Average of two independent replicate chromatins in panel A.

Chr10 Primer sequences:
Forward- TCCTTCTCCCAACAATCAGC
Reverse- GATGTCGCTCCGAATCTTG

Antibodies Used: H3K9me3 (abcam ab8898), H3K9Ac (upstate 07-352), H3K36me3 (abcam ab9050), H3 (abcam ab1791)

Protocol Information

Cell Type: HEK 293T (2) 10cm dishes 70-80% confluent
Total Sample Volume: 300ul
Fixation Time: 1% Formaldehyde, 13 min
Sonicator Amplitude Setting: 70%
Sonication Pulse Rate: 15 seconds On, 45 seconds Off
Total Sonication On Time: 30 min.
Sample Process Temperature: 4°C

1. Crosslink cells by adding 1% Formaldehyde to the media, 13 min 37°C.
2. Stop by adding pHed Glycine to 0.125M.
3. Wash cold PBS.
4. On ice, add PBS on dish (2.5 ml).
5. Scrape, collect in 15 ml tube, spin for 2 min at 800 rpm, 4°C.
6. Resuspend pellet in cellular lysis buffer-protease inhibitors (Example: 2.5 ml for (1) 15cm dish, 1 ml for (2) 10cm dishes)
7. Incubate 5 min on ice and then spin for 2 min at 800 rpm 4°C.
8. Discard the supernatant and resuspend the pellet in nuclear lysis buffer-protease inhibitors (2 ml for four 15 cm dishes, 300 ml for two 10 cm dishes 293T-3 10 cm RPE).
   - Cellular lysis: 5 mM PIPES, 85 mM KCl, 0.5% NP40.
   - Nuclear lysis: 50 mM Tris pH8, 10 mM EDTA pH8, 0.2 or 1% SDS (depends on application).
9. Sonication : For two 10 cm dishes (293T), using Q800R System Sonicate for 30 min at 70%. Sonication test. Spin for 10 min at 14000 rpm, 4 °C to clear chromatin.

Customer Notes:
- To obtain chromatin less than 300bp. Aliquot 300ul of chromatin in thin walled PCR tubes from Brandtech #781312.
RPE cells :
- In 1% SDS : 35 min of sonication time (total on).
- In 0.2% SDS (or other weaker detergent): 45 min of sonication time (total on).

*Total time is longer than a probe alone but 12 samples can be processed at one time and results are very consistent.