Immunoprecipitated PRMTs as an Enzyme Source

Although most recombinant PRMTs retain enzymatic activity in the absence of other proteins or cofactors, it is clearly possible that this basic activity may be enhanced or altered in subtle ways if these mammalian enzymes are expressed in their normal cellular environment. The expression of PRMTs in mammalian cells will bestow them with the correct posttranslational modifications, allow them to more readily fold correctly, and purify with other proteins that regulate their activity. Thus, at times it is necessary to use mammalian expressed PRMTs, which are immunoprecipitated (IPed) to serve as an enzyme source. Usually, PRMTs are fused to a Myc or HA tag, and antibodies against these tags are used to IP the PRMT from transiently transfected mammalian cells. This method facilitates the purification of PRMTs with their associated cofactors and posttranslational modifications. It should be noted that PRMTs (and their substrates) should never be Flag-tagged for *in vitro* methylation experiments. It was reported that Flag M2-agarose enriches for PRMT5 activity (Nishioka and Reinberg, 2003), thus many affinity purified Flag-tagged complexes are "contaminated" with PRMT5, confounding the field. Here, we demonstrate this approach using Myc-PRMT5 as a sample.

1. Seed exponentially growing HeLa cells at 1-2 X 10^6 cells/10-cm tissue culture dish in 10 mL of the appropriate complete growth medium the day prior to transfection.
2. Incubate the cells at 37 ºC and 5% CO_2. The plate should be 80% confluent on the day of transfection.
3. Prepare the DNA-Lipofectamine 2000 complex according to the manufacturer's instructions. In this case we used 12 µg of the pVAX-PRMT5 plasmid.
4. Add DNA-Lipofectamine 2000 complex to the cells and incubate at 37 ºC in a 5% CO_2 incubator for 24–36 hours prior to collecting the cells.
5. Wash the cells twice with ice-cold 1X PBS and place the cell culture dish on ice.
6. Drain the PBS, then add 800 µl ice-cold mild lysis buffer (150 mm NaCl, 5 mm EDTA, 1% Triton X-100, 10 mM Tris-HCl, pH 7.5) with both protease and phosphatase inhibitors.
7. Scrape adherent cells off the dish using a cold plastic cell scraper, then gently transfer the cell suspension into a pre-cooled microcentrifuge tube.
8. Maintain constant agitation for 10 min. at 4 ºC and then sonicate twice in 8 second bursts at 30% amplitude.
9. Centrifuge at 15,000 *g* for 15 min at 4 ºC, keeping the supernatant on ice.
10. To reduce non-specific binding, pre-clear the supernatant by adding mild lysis buffer washed 50 µL protein A/G beads and incubating at 4 ºC for 30 minutes on a rocker or orbital shaker.
11. Remove the protein A/G beads by centrifugation at 15,000 *g* at 4 ºC for 1 min. Transfer the supernatant to a fresh centrifuge tube.
12. Add 3 µL c-Myc antibody to the supernatant and incubate overnight at 4 ºC with rotation.

13. The next day, centrifuge at 15,000 *g* at 4 ºC for 15 minutes and discard pellet and keep supernatant for immunoprecipitation.
14. Wash the 50 μL protein A/G beads twice with ice-cold mild lysis buffer and mix with supernatant and gently rock on either a rocker or orbital shaker for 1 h at 4 ºC to capture the immunocomplex.
15. Collect the beads by centrifugation. Centrifuge for 30 seconds in the microcentrifuge at 8,000 *g*. Discard the supernatant and wash the beads 3 times with 1 mL ice-cold mild lysis buffer.
16. Wash with 1 mL ice-cold 1X PBS three times. In the last wash, re-suspend the beads and aliquot 100 μL into centrifuge tube. It is recommended to cut the tip off of the pipette when manipulating beads to avoid uneven distributions.
17. Centrifuge at 8,000 *g* at 4 ºC for 30 s and then carefully remove the supernatant. The remaining beads contain approximately 100-200 ng Myc-PRMT5 that can be used directly as an enzyme source for *in vitro* methylation reactions. It is not necessary to elute Myc-PRMTs from beads. The purified Myc-PRMT5 will provide enough enzyme for ten reactions described in the protocol for ***In vitro* Arginine Methylation Assays**.

References

Nishioka, K., Reinberg, D., 2003. Methods and tips for the purification of human histone methyltransferases. Methods. 31**,** 49-58.