Using Rabbit Reticulocyte Lysates as an Enzyme Source

The rabbit reticulocyte *in vitro* translation lysates (RRL) is an enzyme "soup" that facilitates *in vitro* transcription/translation (IVTT). PRMT activity has been shown to be present in rabbit reticulocyte lysates (Cheng et al., 2007). Indeed, PRMT1, PRMT5 and CARM1 can all be IPed from reticulocyte lysates. In addition, insect-derived lysates (ICL) and wheat germ extracts (WGE) also harbor robust arginine methyltransferase activity (Denman, 2008). Thus, candidate PRMT substrates can be cloned into expression vectors that are driven by T7 or SP6 promoters, and then subjected to *in vitro* transcription/translation using the RRL system in the presence of tritium-labeled AdoMet. Bona fide substrates will be radio-labeled in this assay.

1. The open reading frame of the substrate of interest is cloned into a vector that is driven by a T7- or SP6- promoter.
2. According to the manufacturer's instructions, mix 12 µL TNT rabbit reticulocyte lysate, 1 µL substrate vector (1 µg/µL), 1 µL S-adenosyl-L-[methyl-^{3}H]methionine (85 Ci/mmol from a 0.5 mCi/ml stock solution; Perkin-Elmer), and other components in the kit to a final volume of 25 µL.
3. Incubate the reaction at 30 ºC for 90 min.
4. Add 5 µL of 6X protein sample loading buffer and heat at 95 ºC for 5 min.
5. Perform remaining steps in ***In vitro* Arginine Methylation Assays**, starting with step 5.

References

Cheng, D., Cote, J., Shaaban, S., Bedford, M. T., 2007. The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. Mol Cell. 25**,** 71-83.

Denman, R. B., 2008. Protein methyltransferase activities in commercial in vitro translation systems. J Biochem. 144**,** 223-33.