

***in vitro* ubiquitination assay for IKAROS by CRBN (positive control)**

	#1	#2	#3	#4	#5	#6
1 ul Substrates (200 uM, IKAROS)	+	+	+	+	+	+
1 ul E3 ubiquitination buffer (20X)	+	+	+	+	+	+
1 ul MG132 (200 uM)	+	+	+	+	+	+
1 ul MgATP (20X)	+	+	+	+	+	+
1 ul DDB1 (20 uM)	+	+	+	+	+	+
1 ul CUL4a (20 uM)	+	+	+	+	+	+
1 ul ROC1 (20 uM)	+	+	+	+	+	+
1 ul E1 (2 uM)	-	+	+	+	+	+
2 ul E2 (10X) *	-	-	+	+	+	+
1 ul E3 (CRBN, 20 uM)	-	-	-	+	+	+
1 ul pomalidomide (10 mM)	-	-	-	-	+	+
1 ul Ubiquitin (20X)	-	-	-	-	-	+
H ₂ O	13 ul	12 ul	10 ul	9 ul	8 ul	7 ul

Incubate at 30° for 2 hours. Mix with 2x loading dye and run for Western blotting—do not boil sample before loading gel!