

Scale-Up Guidelines

	A	B	C	
O/N Required (ml)	1	10	50	
LB inoculated (ml)	10	100	500	
Sonicate (ml)	1	10	4 x 10*	10ml / tube
Glutathione Sepharose (1:1)(μ l)	25	200	4 x 200**	
Reaction mix (μ l)	30	240	2 x 375	
Elution Buffer (ml)	0.5	1	2 x 1.5	

* i.e., split 40 ml to four 30 ml sarstedt tubes

** I use four 15 ml falcon tubes each containing 10 ml of sonicate with 200 μ l of glutathione sepharose. After incubation and washing I combine the beads from two falcon tubes into one eppendorf tube so I am left with two eppendorfs each containing ca. 400 μ l beads.

A= pilot-scale

B= Western Blot

C= Screen Library

This is what I have done for my RB fusions. Different constructs will likely vary with respect to labelling efficiency, solubility, etc. With RB 379-792 construct I typically find that 250,000 cpm = 5-25 μ l eluate.