# RRAS2

**PDB:**2ERY

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**RRAS2A-s001
**Entry Clone Source:**MGC
**SGC Clone Accession:**
**Tag:**Tag removed
**Host:**BL-21(DE3)R3

## Construct

**Prelude:**
**Sequence:**

**Vector:**pNIC28-Bsa4

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**Transformed 50 µl competent BL-21 (DE3) phage resistant cells with 10 µl of the plasmid DNA and plated out onto LB plate plus 50 mg/ml kanamycin. The next day colonies were picked out into fresh deep well blocks containing 1 ml TB + 50 mg/ml kanamycin. These were grown overnight and glycerol stocks prepared by adding 333 ml of 60 % glycerol to 1 ml of cell suspension, mixing and then storing in a -80°C freezer. The glycerol stock was used to innoculate 10 mls of TB + 50 mg/ml kanamycin which was grown overnight at 37°C as a starter culture for a 1 litre growth. The large scale growth was grown at 37°C until approximately 30 min. before induction when the temperature was lowered to 25°C. Protein production was induced with the addition of 1mM IPTG. The next day cells were harvested by centrifugation at 4000 rpm for 15 minutes. The pellet was then stored in the -80°C freezer.

## Purification

**Procedure**
Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham)

Buffers: Affinity Binding Buffer: 10mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP. Affinity Wash Buffer: 50mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP. Affinity Elution Buffer: 250mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP.

## Extraction

**Procedure**
10mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH 2 PO 4, 0.5mM TCEP, 1x complete PI EDTA free tablet/50mls. The pellet (17.33 g) was resuspended with 3x volume of lysis buffer (approximately 120 mls final) by intermitently placing the pellet in a 37°C water bath and vortexing. Once resuspended the cells were (1) broken by one passage through the Constant Systems cell breaker; (2) sonicating; (3) DNA precipitation with the addition of PEI to a final oncentration of 0.15 % for 30 mins on ice followed by a 17,000 rpm at 4°C to remove precipitation; (4) the supernatant was filtered through a GF/0.2 μM serum acrodiscs.
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham)

Buffers: Affinity Binding Buffer: 10mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP. Affinity Wash Buffer: 50mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP. Affinity Elution Buffer: 250mM Imidazole, 300mM NaCl, 50mM pH8.0 NaH2PO4, 0.5mM TCEP.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**