

3

2.5

2

1.5

1

0.5

0

Whole cell

lysate

Mitochondrial

fraction

Endoplamic

reticulam

fraction

- ultracentrifugation using iodixanol (20). Western blots of equal proportions of all the three interfaces in the iodixanol column produced after ultracentrifugation revealed small amount of endoplasmic reticulam contamination which contain very small amount of NSP4 present in interface I, which has higher molecular weight and the purified mitochondrial fraction contain large amount of NSP4 (20 KD) which is present in interface II. Cox 4 was used as mitochondrial marker and calnexin was used as endoplasmic reticulam marker. B Actin was used as cytoplasmic marker.
- (B) MA104 cells were infected with SA11 (2 moi) for 8 hpi and the mitochondrial fraction and endoplasmic reticulam fraction were separated by ultracentrifugation using sucrose gradient (21) followed by western blot analysis and densitometric analysis of same amount of protein from whole cell lysate, mitochondrial fraction, endoplasmic reticulam fraction.
- (C) Subcellular localization of NSP4 using confocal microscopy as described in materials and methods.