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This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

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Product no AS13 2653

CpxA | Conjugative plasmid expression

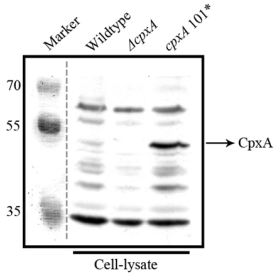
Product information

Immunogen	Recombinant CpxA (residues 26 to 168) over-expressed as a N-terminal fusion with His(x6)-tag, which was removed prior to immunization. The antigen originates from enteropathogenic *Yersinia pseudotuberculosis* YPIII; B1JQV1, NCBI annotated locus tag YPK_4133.
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl of sterile water
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Additional information	The strains were grown in LB broth until late stationary phase (absorbance at 600nm of ~1,5), To avoid interference from cross-reacting bands, aim to maximize protein separation with the region of 35 to 55 kDa, CpxA could be routinely detected from a 20 µL of sample that was derived from 1ml of pelleted bacteria resuspended in 200 µl of 1x loading buffer, Re-use of diluted working strength antibody is not recommended

Application information

Recommended dilution	1 : 2000 (WB)
Expected | apparent MW	51,8 | 52-53 kDa
Confirmed reactivity	*Yersinia pseudotuberculosis*
Not reactive in	No confirmed exceptions from predicted reactivity are currently known

application example



Aliquots of 20 µL total cell lysate, derived from 1 ml of pelleted *Y. pseudotuberculosis* YPIII bacteria (absorbance at 600 nm of ~1.5) that was extracted with 200 l 1x loading buffer, were separated by 12 % acrylamide SDS-PAGE and then wet-blotted for 1h to Immobilon®-P PVDF membrane (Millipore). Following transfer, the membrane was blocked immediately in milk solution (10 % milk powder in TBST) for 1 h at room temperature with agitation. Membrane was incubated in the primary antibody at a dilution of 1:2000 overnight at 4 °C with agitation. The antibody solution was decanted and the membrane was washed twice for 15 min in TBST at room temperature with agitation. The membrane was incubated in secondary antibody (anti-rabbit IgG conjugated horse radish peroxidase and sourced from GE Healthcare) diluted to 1:10000 for 1h at room temperature with agitation. The membrane washed as above for 45 min and then CpxA detected with homemade ECL detection reagent. Exposure time was 180 seconds.

Lanes: **Marker:** PageRuler™Plus Prestained Ladder (ThermoScientific)

Wildtype: *Y. pseudotuberculosis* YPIII/pIB102

***cpxA*:** *Y. pseudotuberculosis* YPIII07/pIB102 (*cpxA* in frame deletion of codons 41 to 449) [Carlsson *et al.,* Infect. Immun., 75 (2007), pp. 3913–3924]

***cpxA101**:* *Y. pseudotuberculosis* YPIII/pIB102 (*cpxA* allele encoding for the substitution of T253P) [Liu *et al.,* PLoS One, 6 (2011), p. e23314]

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