

Antibody Delivery Reagent with HVJ-Envelope (HVJ-E) into living cells

# GenomONE™ - CAB

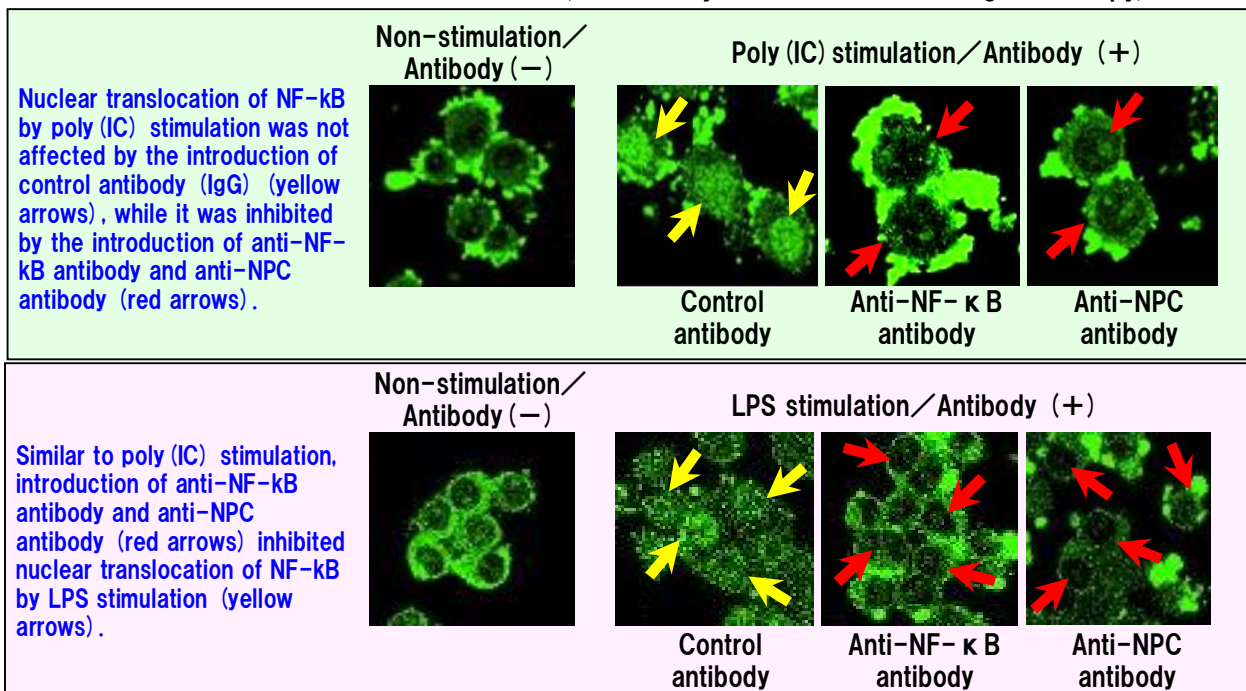
For functional analysis of intracellular proteins!

Functional analysis following intracellular antibody delivery

See back side for the protocol for each experiment.

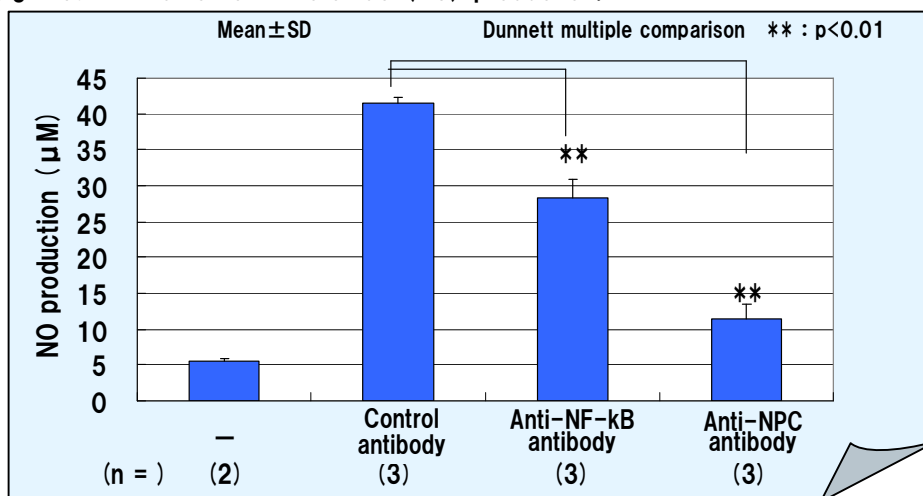
## 【Experiment 1】 Inhibition of nuclear translocation of NF-κB

Introduction of anti-NF-κB antibody or anti-NPC (nuclear pore complex protein) antibody to Raw 264.7 cells inhibited nuclear translocation of NF-κB (observed by confocal laser scanning microscopy).



## 【Experiment 2】 Inhibition of Nitric Oxide production

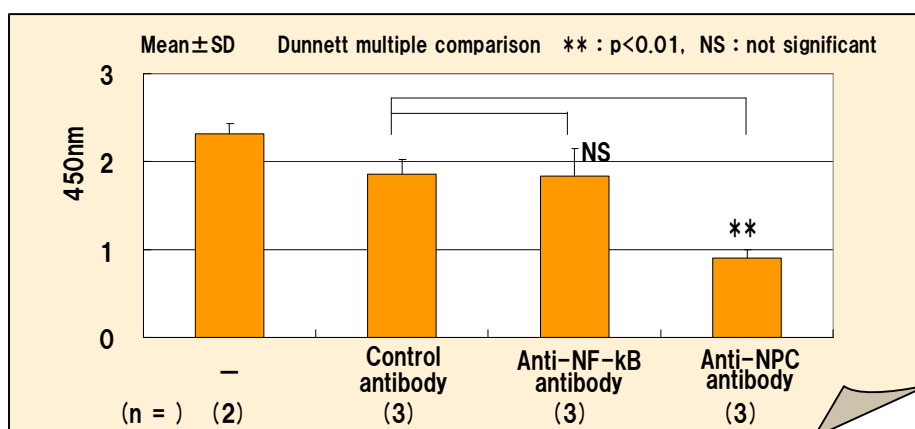
Introduction of anti-NF-κB antibodies or anti-NPC (nuclear pore complex protein) antibodies to Raw 264.7 cells led to significant inhibition of nitric oxide (NO) production.



Reference related to GenomONE-CAB : Kondo Y. et al., J. Immunol. Methods, 332,10-17 (2008).

## 【Experiment 3】 Inhibition of cell growth

Introduction of anti-NPC antibody to Raw264.7 cells resulted in significant inhibition of cell growth. On the other hand, introduction of anti-NF- $\kappa$ B antibody did not produce inhibition.



### 【Protocol / Experiment 1】

- Raw264.7 cells (ATCC TIB-71)  $1-3 \times 10^4$  cells/well, 10%FBS-DMEM (8-well chambered coverglass; Nunc155411)  
 ↓ delivery of 0.8-1  $\mu$ g/well of Control antibody (Sigma, M9269), anti-NF- $\kappa$ B antibody (Santa Cruz Biotechnology Inc., Sc-8008) or anti-NPC antibody into cells using *GenomONE-CAb*  
 ↓ incubation for 2-3 hr at 37°C  
 ↓ addition of Poly (IC) (final 100  $\mu$ g/mL) or LPS (10  $\mu$ g/mL), 200  $\mu$ L/well  
 ↓ incubation for 30 min 37°C (15 min for LPS-treated group)  
 ↓ wash the cells with cold 10%FBS-DMEM followed by cold PBS (-)  
 ↓ fix the cells (4% PFA; for 15 min, r.t. → PBS wash → 0.2% Triton X-100; for 5 min, r.t. → PBS wash → 3%BSA/PBS; for 10 min, r.t.)  
 ↓ Rabbit anti-NF- $\kappa$ B p65 (c) antibody (kindly provided by Immuno-Biological Laboratories Co., Ltd., #8883); 0.4-4  $\mu$ g/mL, 3% BSA solution  
 ↓ incubation for 1 hr at r.t.  
 ↓ wash the cells with PBS (-) (3 times)  
 ↓ AlexaFluor 488-Goat Anti-Mouse IgG, F(ab')<sub>2</sub> fragment, 1/500 dilution (Invitrogen, A11017)  
 ↓ wash the cells with PBS (-) (3 times)  
 ↓ observation by confocal laser scanning microscopy

(This experiment was performed as joint research with Immuno-Biological Laboratories Co., Ltd. We express our heartfelt thanks to Dr. Kiyoshi Ishikawa and the other individuals involved.)

### 【Protocol / Experiment 2 & 3】

- Raw264.7 cells (ATCC TIB-71)  $4 \times 10^4$  cells/well, 10%FBS-DMEM (96-well plate; IWAKI 3860-096), overnight incubation  
 ↓ delivery of 0.8-1  $\mu$ g/well of Control antibody (Sigma, M9269), anti-NF- $\kappa$ B antibody (Santa Cruz Biotechnology Inc., Sc-8008) or anti-NPC antibody into cells using *GenomONE-CAb*  
 ↓ incubation for 2-3 hr at 37°C  
 ↓ 【Exp.2】  
 ↓ addition of LPS (10  $\mu$ g/mL), 200  $\mu$ L/well  
 ↓ incubation for 19 hr at 37°C  
 ↓ supernatant is added to each well of another 96-well plate; 150  $\mu$ L/well  
 ↓ Griess Reagent ( $\times 2$  conc.), 50  $\mu$ L/well  
 ↓ Left to stand for 10 min at r.t.  
 ↓ A540nm
- 【Exp.3】  
 ↓ addition of Cell Count Reagent SF (nacalai tesque; 07553-44) 20  $\mu$ L/well  
 ↓ incubation for 2-3 hr at 37°C  
 ↓ 450nm

### Specifications of *GenomONE-CAb*

Cat. #	Freeze-dried HVJ-E (Equivalent to 0.26 mL/vial)	Reagent I (Equivalent to 0.26 mL/vial)	Reagent II (0.3 mL/vial)	Reagent III (1 mL/vial)	Buffer (6.5 mL/vial)
AB001	1	1	1	1	1
AB004	4	4	1	4	1