

## Retrovirus Restriction Factor Detection Set

**CATALOG No.: PSI-1817**

### BACKGROUND:

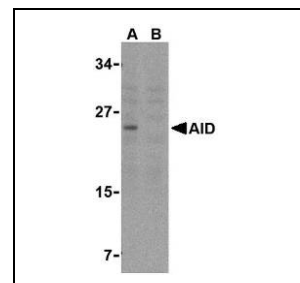
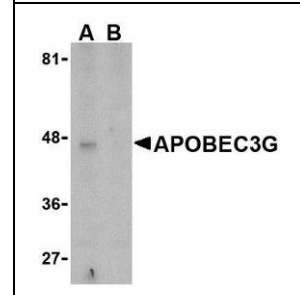
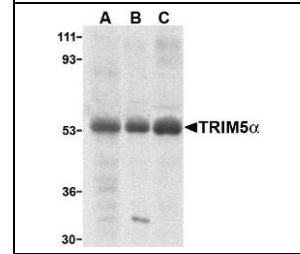
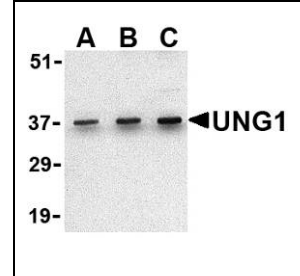
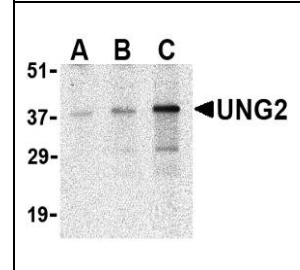
Mammalian cells have developed multiple strategies to limit retroviral infection including numerous proteins termed restriction factors that restrict retrovirus replication and infection. One such protein is TRIM5, a member of a broad family of otherwise unrelated proteins (1) whose longest isoform, TRIM5 $\alpha$ , enables resistance to infection by HIV-1 (2,3) through rapid degradation of HIV-1 Gag polyproteins (4). Another protein, APOBEC3G (and to a lesser extent APOBEC3F) can be incorporated into HIV-1 virions and induce hypermutation in the newly synthesized viral DNA and thus destabilize the viral genome (5,6). This innate mechanism of retroviral resistance is counteracted by the HIV-1 Vif protein by inducing the ubiquitization and degradation of APOBEC3G; a single amino acid substitution (D128K) blocks APOBEC3G depletion without affecting its inhibitory activity (7). The human uracil-DNA glycosylase UNG2 (8) can also be incorporated into the HIV-1 virion, indicating that it is required to remove uracils from the viral genome (9). It has been suggested that the UNG2 contributes to the APOBEC3G-mediated loss of infectivity by generating abasic sites in the viral genome (10). UNG1, the mitochondrial form of UNG, is transcribed from the same gene as UNG2 through differentially regulated promoters and alternative splicing (8), but does not appear to have anti-retroviral properties (11). AID, a protein related to APOBEC3 (12) also possesses cytidine deaminase activity that can be blocked by the HIV-1 Vif protein in *E. coli* (13), but so far it appears unlikely that AID deaminates dC to dU residues in HIV cDNA as does APOBEC3G (5).

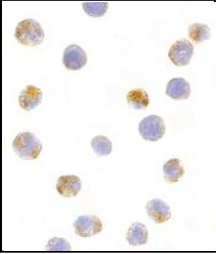
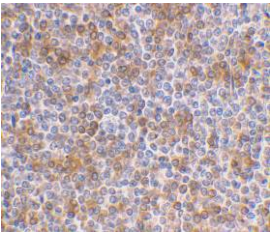
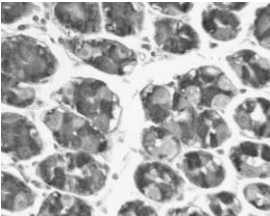
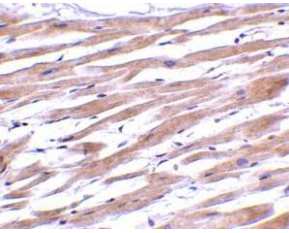
### KIT CONTENTS:

AID antibody, **Catalog No. 3091 (50 $\mu$ g)**.  
 APOBEC3G antibody (NT), **Catalog No. 3257 (50 $\mu$ g)**.  
 TRIM5 $\alpha$  antibody (IN1), **Catalog No. 3247 (50 $\mu$ g)**.  
 UNG1 antibody (CT), **Catalog No. 3863 (50 $\mu$ g)**.  
 UNG2 antibody (NT), **Catalog No. 3859 (50 $\mu$ g)**.

### SOURCE:

Rabbit polyclonal antibodies were raised against peptides corresponding to amino acid sequences from each of the corresponding proteins.

	<p>Western blot analysis of AID in Ramos whole cell lysate with AID antibody at 2 <math>\mu</math>g/ml in either the (A) absence or (B) presence of blocking peptide.</p>
	<p>Western blot analysis of APOBEC3G expression in Caco-2 cell lysate in the (A), absence and (B) presence of blocking peptide with APOBEC3G antibody at 5 <math>\mu</math>g/ml.</p>
	<p>Western blot analysis of TRIM5<math>\alpha</math> expression in human stomach (A), thymus (B), and uterus (C) cell lysate with TRIM5<math>\alpha</math> antibody at 2 <math>\mu</math>g/ml.</p>
	<p>Western blot analysis of UNG1 in C2C12 cell lysate with UNG1 antibody at (A) 0.5, (B) 1 and (C) 2 <math>\mu</math>g/ml.</p>
	<p>Western blot analysis of UNG2 in mouse bladder tissue lysate with UNG2 antibody at (A) 0.5, (B) 1 and (C) 2 <math>\mu</math>g/ml.</p>

	Immunocytochemistry of AID in Ramos cells with Ramos antibody at 10 µg/ml.
	Immunohistochemistry of human spleen using APOBEC3G antibody at 1 µg/ml.
	Immunohistochemistry of human stomach using TRIM5α antibody at 2 µg/ml.
	Immunohistochemistry of UNG1 in human heart tissue with UNG1 antibody at 2 µg/ml.

**APPLICATION:**

These polyclonal antibodies can be used for detection of AID, APOBEC3G, TRIM5α, UNG1 and UNG2 by immunoblot at 1 – 5 µg/ml. These antibodies can also be used at 1 – 10 µg/ml to detect their respective proteins via immunohistochemistry / immunocytochemistry. **For research use only.**

**STORAGE:**

Antibodies are supplied as affinity chromatography purified IgG in PBS containing 0.02% sodium azide. Antibodies should be stored at -20°C.

**POSITIVE CONTROLS:**

AID antibody: Ramos Lysate, **Catalog No. 1225.**

APOBEC3G antibody: Caco-2 Cell Lysate, **Catalog No. 1223.**

TRIM5α antibody: Human Uterus Tissue Lysate, **Catalog No. 1317.**

UNG1 antibody: C2C12 Cell Lysate, **Catalog No. 1285.**

UNG2 antibody: Mouse Bladder Tissue Lysate, **Catalog No. 1410.**

**RELATED PRODUCTS:**

AID peptide, **Catalog No. 3091P.**

APOBEC3G peptide (NT), **Catalog No. 3257P.**

TRIM5α peptide (IN1), **Catalog No. 3249P.**

UNG1 peptide (CT), **Catalog No. 3863P.**

UNG2 peptide (NT), **Catalog No. 3859P.**



#### REFERENCES:

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3. Hatzioannou T, Perez-Caballero D, Yang A, et al. Retrovirus resistance factors REF1 and Lv1 are species-specific variants of TRIM5 $\alpha$ . *Proc. Nat'l. Acad. Sci. USA* 2004; 101:10774-9.
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7. Xu H, Svarovskaia ES, Barr R, et al. A single amino acid substitution in human APOBEC3G antiviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. *Proc. Natl. Acad. Sci. USA* 2004; 101:5652-7.
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9. Willetts KE, Rey F, Agostini I, et al. DNA repair enzyme uracil DNA glycosylase is specifically incorporated into human immunodeficiency virus type 1 viral particles through a Vpr-independent mechanism. *J. Virol.* 1999; 73:1682-8.
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12. Cascalho M. Advantages and disadvantages of cytidine deamination. *J. Immunol.* 2004; 172:6513-8.
13. Santa-Marta M, Da Silva FA, Fonseca AM, et al. HIV-1 Vif protein blocks the cytidine deaminase activity of B-cell specific AID in *E. coli* by a similar mechanism of action. *Mol. Imm.* 2007; 44:583-90.

#### WESTERN BLOT PROTOCOL:

- 1) Load 20 to 25 micrograms of whole cell lysate per lane in an SDS-PAGE mini gel.
- 2) Run at 20 mA per gel until the dye front is close to the bottom.
- 3) Transfer the proteins to a nitrocellulose membrane at 250 mA in transfer buffer for 1-4 h, depending on the size of the target protein.
- 4) Incubate the blot with blocking buffer (5% non-fat dry milk in TBS) overnight at 4°C or 2 hr at room temperature (RT).
- 5) Incubate the blot with primary antibody (diluted 1:250 to 1:1000 in blocking buffer) for 1 hr in blocking buffer at RT.
- 6) Wash the blot 3 x 10 min in washing buffer (TBS containing 0.1% Tween 20) with shaking.
- 7) Incubate blot with anti-rabbit IgG-HRP conjugate (diluted 1:10,000 -1:20,000 in blocking buffer) for 1 h in blocking buffer at RT.
- 8) Wash 3 x 10 min in washing buffer with shaking.
- 9) Drain washing buffer, add ECL solution and develop for 1 min.
- 10) Expose to X-ray film for 1 to 30 min.

#### MATERIALS NEEDED:

Nitrocellulose membrane  
Non-fat dry milk  
Tween-20  
Antibody detection kit

#### TBS:

- 125 mM NaCl
- 25 mM Tris pH 8.0
- 0.1% Tween 20

#### SDS/Running Buffer:

- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS

#### Transfer Buffer:

- 20 mM Tris
- 150 mM Glycine
- 20% methanol
- 0.038% SDS