ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Human AKT1 (RAC-PK Alpha) Protein Controls

½ **Cat.** AKT12-C	**Human Akt-1** protein (phosphorylated at Thr308 and Ser473) WB +Ve control	**SIZE**: 100 ul
½ **Cat.** AKT13-C	**Human Akt-1** protein (inactive, non-phosphorylated) WB +Ve control	**SIZE**: 100 ul

Putative human homolog of the protooncogene v-akt of the acutely transforming retrovirus AKT8 have been cloned. These protein-serine/threonine kinase proteins have a catalytic domain closely related to both PKA and PKC and have been designated **rac** (related to A and C kinases), **pkb** (Protein kinase B) or **Akt**. RAC protein kinase family members feature pleckstrin homology (PH) domain at the amino terminus and a protein-serine/threonine kinase catalytic domain at the carboxy terminus. The Amino terminal domain (referred to as AH-Akt Homology domain) spans from 1-148 amino acids and contains the PH domain, a region found in diverse group of signaling proteins. The PH domain (amino acids 1-106) has been implicated in interactions with other proteins such as G-protein ▪ ▪ subunits, as well as phosphoinositides. The kinase domain is located between residues 148 to 411. These enzymes are activated by diverse ligands such as PGDF, EGF and basic FG in NIH 3T3, Rat-1 or Swiss-3T3 cells.

Source of AKTs

Human AKT1, fusion protein **~60 kDa** (protein accession # BC000479. his-tag at the N-terminus) was expressed in sf21 cell and purified >95%. This preparation was active (specific activity 300-1800 Units/mg). AKT1 was activated with MAPKAP kinase 2 and PDK1 and repurified. For **western blot +ve control (Cat # AKT12-C)**, it is supplied in SDS-PAGE sample buffer (reduced). This preparation of **AKT1 is phosphorylated but not active** in sample buffer. Activation of AKT1 involves (and requires) phosphorylation of serine 473. This kinase is phosphorylated at Thr308 and Ser473. This protein has an amino terminal polyhistidine tag and had been purified by Ni-NTA agarose chromatography to > 95% by SDS-PAGE. Load ~10 ul/lane to visualize with appropriate antibodies. Store at –20oC in suitable aliquots. Avoid repeated thawing or heating.

Human AKT1, fusion protein **~60 kDa** (his-tag at the N-terminus) was expressed in sf21 cell and purified >90%. This preparation of **AKT1 is inactive or minimally active**. It has not been phosphorylated or de-phosphorylated. It may have some basal level of phosphorylation. For **western blot +ve control (Cat # AKT13-C)**, it is supplied in SDS-PAGE sample buffer (reduced). Load ~10 ul/lane to visualize with appropriate antibodies. Store at –20oC in suitable aliquots. Avoid repeated thawing or heating.

Storage

Short-term: unopened, undiluted vials for less than a week at 4oC.

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder.

Suggested uses

Both **AKT12-C and AKT13-C** are recommended for Western blots only to study the reactivity of antibodies with the phosphorylated or non-phosphorylated forms of AKT or to study crossreactivity of antibodies. Due to the presence of SDS-APGE buffer in the controls, these are not suitable for ELISA or IP or other applications.

General References: (1) Konishi et al. (1994) BBRC **205**, 817-825; Coffer & Woodgett (1991) Eur. J. Biochem. **201**, 475-481; Jones et al. (1991) PNAS USA **88**, 4171-4175; Marte BM & Downward J (1997) TIBS 22, 355;

*This product is for *in vitro* research use only.

Related material available from ADI:

Western Blot recycling kit (Use the same blot to probe with multiple antibodies CSP11, CLO11, etc.) **recycle blot at room temp in 5-10 min**; No mercaptoethanol or heating required).

AKT12-13-C 150625A

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