REF. Number : ATGKF008

NK VUE TUBE

FOR *IN VITRO* DIAGNOSTIC USE
BLOOD COLLECTION TUBES TO BE USED
WITH NK VUE ELISA

1. INTENDED USE

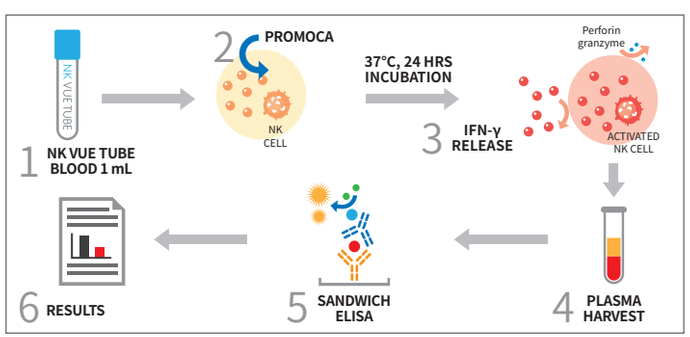
The NK VUE tubes are blood collection tubes to be used in conjunction with NK VUE ELISA. NK VUE is intended for *in vitro* diagnostic use, for the monitoring of the immune status of individuals. Measurement of NK cell activity could be a useful tool for assessing changes in immunosurveillance, which, in turn, could be indicative of a condition or disease where NK cell activity has been shown to be affected.

2. SUMMARY AND EXPLANATION OF NK VUE

2.1 Principle of NK VUE

NK VUE employs a proprietary stabilized immunomodulatory cytokine (Promoca) to stimulate NK cells in whole blood. After their activation, a quantitative sandwich enzyme immunoassay (ELISA) is used to determine the levels of IFN-γ secreted. To this end, an anti-IFN-γ monoclonal antibody has been pre-adsorbed on a micro well plate. Samples are pipetted into the wells and IFN-γ allowed to bind to the immobilized antibody. After washing away all unbound material, a second anti-IFN-γ monoclonal antibody complexed to an enzyme (HRP) is added to the wells. Following a final wash to remove any unbound antibody-HRP complex, the substrate solution is added to the wells and color is allowed to develop. Absorbance at 450 nm is measured, and the amount of IFN-γ released by the NK cells is finally quantitated by comparison to an IFN-γ standard curve.

Each NK VUE Tube is intended for one clinical sample.



Principle of NK VUE

2.2 Time required for performing blood collection, blood culture, and sample preparation

- Blood collection and culture set up: ~15 min.
- Blood culture, carried out in NK VUE tubes, at 37 °C: 20-24 hours (overnight).
- Sample preparation: ~15 min (recovery of cultured plasma from an NK VUE tube, transfer and storage).

3. PRODUCT AND STORAGE

3.1 Components

Component	Quantity	Feature	Storage	Expiration Date	
				Unopened	Opened
NK VUE Tube	50 tubes	clear liquid in heparinized tube (~0.1mL)	2-8 ℃	12 months from manufacturing	N/A

3.2 Materials required but not provided

- Standard 37 °C incubator
- Either automatic micropipettes (P200 and P1000, or similar) fitted with disposable pipette tips, or disposable plastic Pasteur pipets
- 1.5 mL conical polypropylene microcentrifuge tubes (Eppendorf type) (if needed)
- Microcentrifuge, or clinical centrifuge (if needed)

4. PRECAUTIONS

- For *in vitro* diagnostic use only.
- Use only after fully reading and understanding these guidelines.
- Handle human blood as if potentially infectious. Observe relevant blood handling guidelines. Wear eye protection, disposable gloves and wash hands thoroughly after use.
- When opening the lid/cap of the tube/vial, or when removing the contents, use GLP procedures, trying to avoid microbial contamination.
- DO NOT use expired NK VUE Tubes.
- For diagnostic use only. Each NK VUE Tube is intended for a single blood collection event. Do not mix or combine samples from different tubes.
- When pipetting samples, use new disposable tips and regularly calibrated pipettes.
- Discard solid waste and biological samples in accordance with Local, Provincial, and Federal regulations.
- Follow general laboratory safety guidelines.

5. TEST PROCEDURE

Blood has to be collected into NK VUE Tubes and promptly incubated at 37 °C. NK VUE Tubes contain an engineered stimulatory cytokine, called Promoca. During the incubation process, IFN-γ will be secreted by NK cells into plasma, and subsequently quantitated using an ELISA assay kit (NK VUE ELISA, available separately).

5.1 Step 1 – Collection and culture of blood, and recovery of induced plasma

A dedicated vacuum blood collection tube (i.e., NK VUE Tube) is used to collect blood directly from the patient. NK VUE Tubes contain Promoca, and heparin as anticoagulant. After blood collection, the tube contents have to be gently mixed and incubated for 20–24 hours at 37 °C in order to elicit the appropriate response from NK cells.

1. Remove the NK VUE Tube from the refrigerator just before use for blood collection. Verify expiration date, and write patient's name, date, and collection time on the space provided on the tube's label.
   - If the NK VUE Tube is left at room temperature for more than 5 min, discard and use a new cold tube.
2. Draw 1 mL of whole blood into the NK VUE Tube using a tube holder and a 21G needle (direct needle blood collection system; e.g., BD catalog #368650). After sample appears to be completely collected (i.e., blood flow stops), continue for a few additional seconds to ensure that the correct volume is drawn. Then, remove the NK VUE Tube from the tube holder and, before mixing the tube contents, hold the tube vertically to verify that the level of blood falls within the limits of the mark on the left side of the tube's label (black vertical rectangle).
   - If collected blood does not amount to, or exceeds it, discard and immediately repeat blood collection using a new tube (taking advantage of the same venipuncture point). If the second tube also fails in collecting the right volume, a new venipuncture point will have to be used. If for any reason a direct needle blood collection system cannot be used, the following instructions must be considered: If a syringe is used, briefly spin NK VUE Tube beforehand to collect contents at its bottom, and remove NK VUE Tube cap/stopper assembly. Then, slowly transfer 1 mL blood into the NK VUE Tube, as to avoid damaging cells during transfer, and re-cap tube firmly. If a butterfly needle needs to be used, it is important to first purge the tubing by collecting some blood into a heparinized blood collection tube (4–5 drops) (do NOT use any other type of blood collection tube, like citrate or EDTA, etc., for this pre-draw). Immediately following the pre-draw, switch to the NK VUE Tube. This process will ensure collection of the proper volume into the NK VUE Tube, calibrated to draw 1 mL of whole blood.
3. Mix the collected blood by gently rolling and inverting the tube 10 times, ensuring that tube walls have been entirely soaked in blood and contents is thoroughly mixed and homogeneous.
   - DO NOT shake nor use a tube roller for mixing as it can affect activation of NK cells.
4. Start incubation at 37 °C as soon as possible (within 15 min), placing the tube UPRIGHT within the incubator.
   - DO NOT refrigerate or freeze blood samples collected into the NK VUE Tubes before culture.
   - Once incubation has started, do not remove tube from incubator unless it's just for a few seconds.
5. The next day, after incubating the NK VUE Tube with blood for 20-24hs at 37 °C, the plasma sample needs to be prepared to be sent to a clinical laboratory for analysis, as follows:
   a. If the upper plasma phase looks mostly clear and yellowish, store the unopened NK Vue Tube in the refrigerator until shipped to the laboratory (between +2 to +8°C for up to 3 days). Alternatively, gently remove the cap from the NK VUE Tube, carefully collect the clear supernatant only, and transfer into a 1.5 mL microcentrifuge tube. The presence of some residual red blood cells is acceptable. Samples can be shipped immediately (at +2 °C to 8 °C) to the lab for analysis. If that is not possible, they can be kept refrigerated at +2°C to 8°C (for up to 3 days), or frozen at -20°C (for up to 3 months), until shipped.
   b. If the upper plasma phase looks pink, the possibility of hemolysis should be assessed by any of the two following ways:
      i. Gently remove the cap from the NK VUE Tube, carefully collect the plasma, transfer it into a 1.5 mL microcentrifuge tube, and centrifuge at 11,500g for 1 min at room temperature, or
      ii. Centrifuge the whole NK VUE Tube at 1,200g for 4 minutes in a clinical centrifuge.
      If plasma still looks pink after centrifugation, a significant degree of hemolysis took place and sample must be discarded (hemolysis can lead to inaccurate results). If plasma becomes yellowish with no signs of pink color after centrifugation, hemolysis can be ruled out and supernatant can be transferred into a 1.5 mL microcentrifuge tube, and shipped or stored as detailed in a. above.
   c. If upper plasma phase looks pink and no centrifuge is available, do not open the NK VUE Tube. Refrigerate it between +2 and +8 °C and ship to lab immediately or within 3 days.
6. Always ensure that tubes containing stored samples are capped properly since partial evaporation of samples will cause inaccurate results.

5.2 Step 2 – IFN-γ ELISA assay

The NK cell activity is measured as IFN-γ released into the supernatant with the NK VUE ELISA, available separately.

6. LIMITATIONS

Results will not be reliable if all the procedures described in this package insert were not properly followed.

7. TECHNICAL SERVICE

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