# KJELDAHL Method

The Kjeldahl Method may be broken down into three main steps:
**Digestion**
**Distillation**
**Titration**

**Step 1 - Digestion**
The goal is to break down the bonds that hold the polypeptides together and convert them into simpler molecules (such as water, carbon dioxide and ammonium sulphate). These reactions can be speeded up by the temperature used during digestion (the higher the temperature used, the faster the digestion can be obtained) and by the presence of acid, salt and catalysts (selenium, copper, mercury, titanium). Vapors that escape from the tubes are aspirated through the suction cap by a JP recirculating water vacuum pump and eliminated in an SMS scrubber. This configuration optimizes the efficiency of the operation. Avoid using digesters without an exhaust system: this will dramatically shorten its life and might cause damage.
This is the most time-consuming step of the analysis.

- If the sample is solid, weigh out approximately 1 - 3 g of the sample in a VELP weighing boat (nitrogen-free) (CM0486000 or CM0486001) and record the weight (the particle size of the sample should be reduced to < 1 mm, for better results. The sample might need to be homogenized, before any operation).
  If the sample is liquid, measure the volume with a pipette and place it in a beaker and stir it using one of VELP's heating magnetic stirrers. If necessary, remove any $CO_2$ (e.g. fizzy drinks) before measuring the volume.

- Place the sample into a VELP glass test tube (where nitrogen content could be quite low, larger sample amounts need to be used) along with 12 - 20 ml of concentrated sulfuric acid, as specified in the method. The total amount of acid needed during a digestion can vary from one sample type to another. Another factor to consider is the loss of acid that occurs due to the evaporation through the exhaust system used. The VELP exhaust system and heat shield control acid loss (around 1.2 ml acid per sample).
  - ✘ A problem that might occur during the digestion is the drying out of the digested sample, a process called "the salting out effect", due to the too high flow rate.

- Add catalyst tablets (select the correct variety according to the protocol):
  VELP Kjeltabs - ST: potassium sulfate, selenium (CT0006609)
  VELP Kjeltabs - W: sodium sulfate, copper sulfate, selenium (CT0006613)
  VELP Kjeltabs - TCT: potassium sulfate, copper sulfate, titanium dioxide (CT0006621)
  VELP Kjeltabs - CM: potassium sulfate, copper sulfate (CT0006650)
  VELP Antifoam - S: sodium sulfate, silicone (CT0006600)

  - ☞ Copper is satisfactory but very slow to react; environmentally friendly
  - ☞ Selenium catalysts usually react very fast and are typically used for substances that are resistant to digestion (e.g. fats and oils)


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- Select the program from the menu (on DKLs the most used applications are pre-installed and others are user-programmable). Just by pressing Menu, Programs, you can choose which Standard Program to select or create a new Customizable Program.

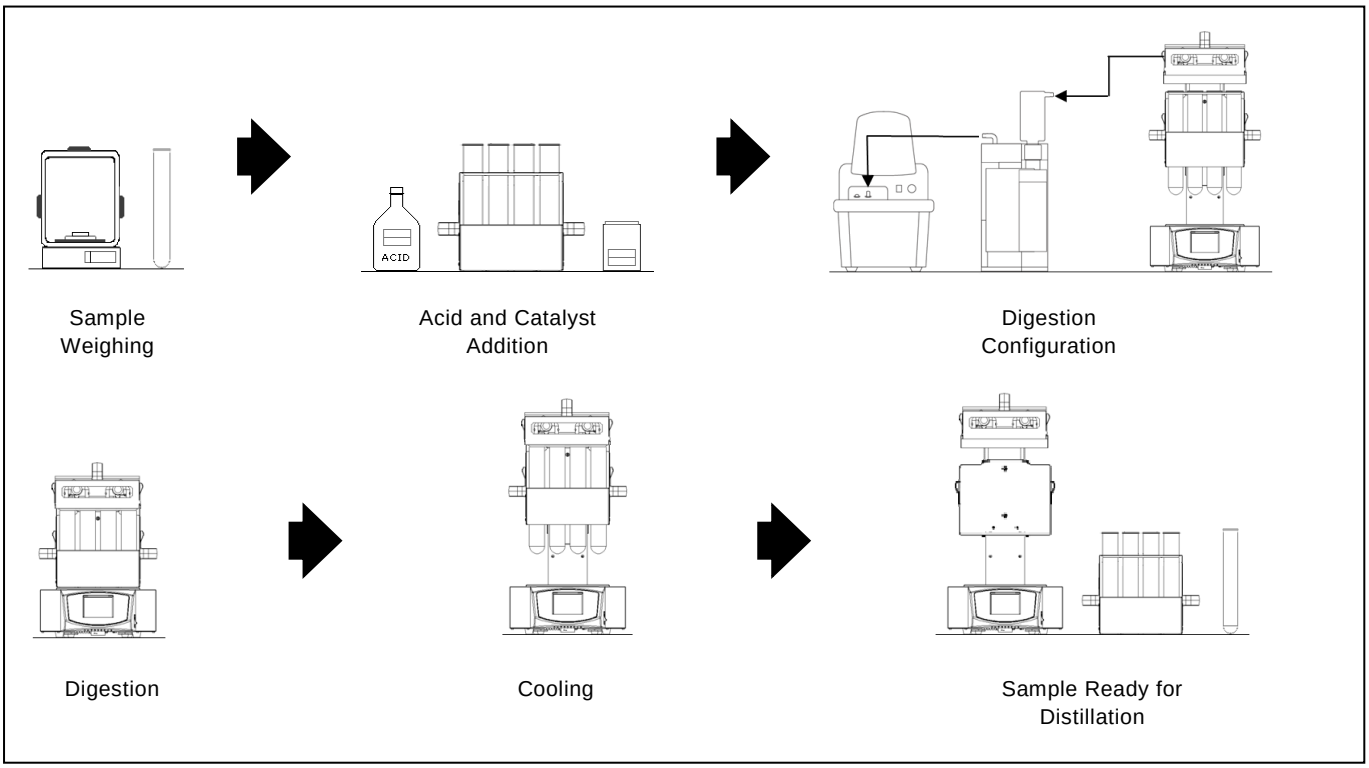
- Lower the samples (automatically on DKLs) into the aluminum heating block (maintenance-free and highly durable) and heat the mixture to the temperature indicated in the Standard Method. (The DKL aluminum heating block ensures the best possible homogeneity across all tubes and a complete digestion in each tube. It can reach 450 °C / 842 °F, ensuring a nitrogen recovery higher than 99% in the following stages).

- Heat the mixture for the time indicated in the Standard Method in order to obtain a clear and colorless solution. During this phase the sulfuric acid reacts with the sample, converting all nitrogen in organic form into inorganic form that is stable and ready to be analyzed.
  - ✖ If any problem occurs during the process and/or if the sample preparation was not correct, the customer can notice the presence of carbon residues (black-brown colored) in the digested mixture and on the walls of the tubes. These are symptoms of an incomplete mineralization of the sample which cannot be processed further.

- Raise the samples (automatically on DKLs) and cool by natural radiation.

- Separate the suction cap (press the up arrow on DKLs) - a drip tray needs to be introduced below the suction cap to collect any drops of acid that might fall from the suction cap glass bells.

Now the tube rack can be removed and the samples are ready to be moved to the distillation phase.





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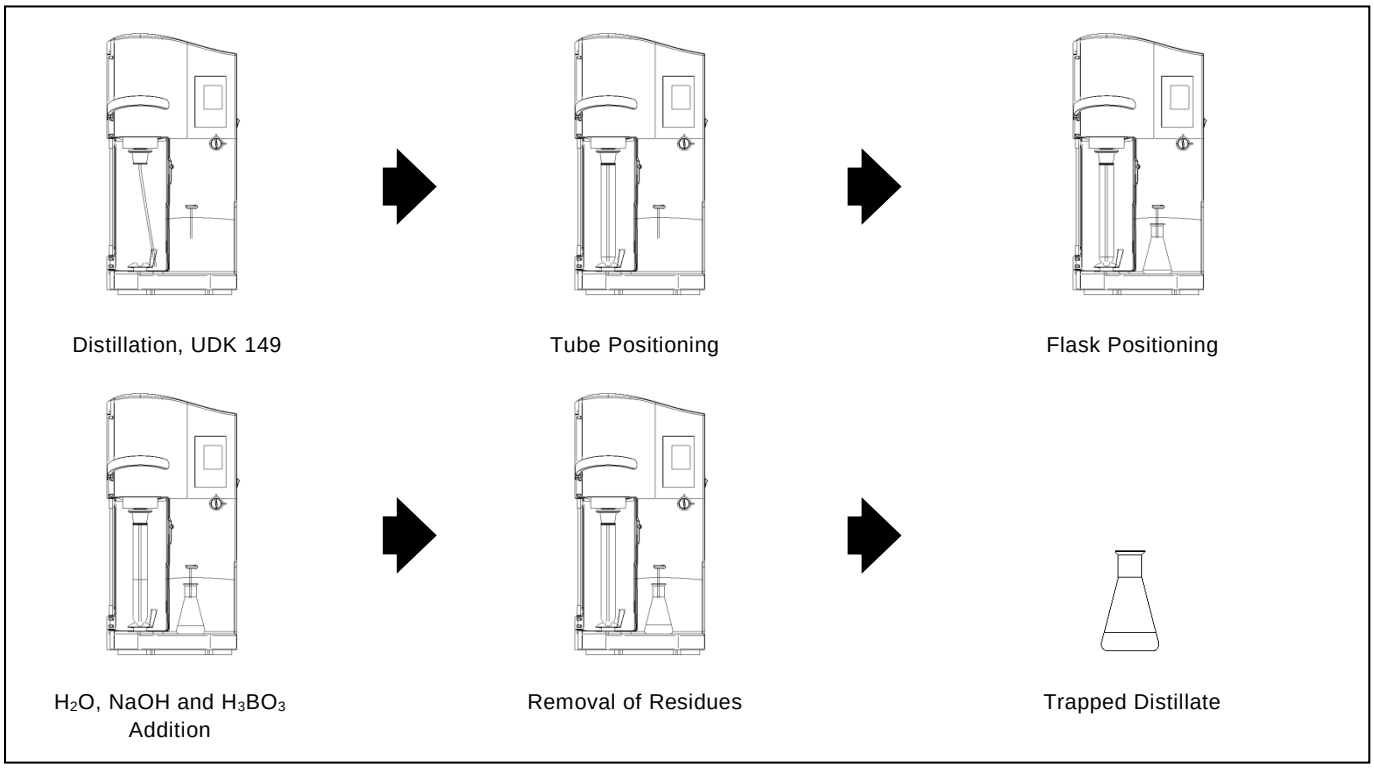
**Step 2 - Distillation**

The ammonium sulphate present in the digested sample are converted into ammonia gas, heated and distilled. The ammonia gas is led into an acid trapping solution where it dissolves and becomes a trapped ammonium ion once again.

Using the Kjeldahl method, nitrites and nitrates are not detected. In order to quantify these elements, a reduction of the sample is necessary (using Devarda alloy) before the digestion stage.

- Add distilled or deionized water to the test tube containing the digested sample to dilute it (automatically on UDK 139, 149, 159). In this way it's easier to detect all the ammonia.
- Separate the nitrogen from the digested mixture by steam distilling (steam output regulation 10-100% on UDK 139, 149, 159), in order to extract ammonia from the alkaline solution.
- Raise the pH of the digested mixture using sodium hydroxide (35%) (automatically on UDKs) to convert $NH_4^+$ (in solid format) into $NH_3$ (gaseous), that will be detected with titration.
- Trap the distilled vapors in a dedicated solution of 25-30 ml of boric acid (automatically on UDK 149, 159) to trap all the nitrogen, eliminating the risk of loss.
- Drain the test tube with the digested sample (automatically on UDK 139, 149 and 159).

Now perform the final titration of the ammonia distilled from the sample, considering that if the nitrogen content of the sample is high, a high-concentrated acid for the titration is needed. Another solution is reducing the quantity of the sample used for the analysis, but in some cases it may cause errors giving wrong results.





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**Step 3 - Titration**

The goal is to determine the amount of ammonia distilled off from the digested solution and hence calculate the nitrogen or protein amount, as %.

☞ ...with UDK 159 (distillation and colorimetric titration unit):

- Add two indicators to the boric acid (4%) solution, in order to follow the titration process by a color change. The color is red in absence of ammonia, turns green in case of significant amount and grey / pink at the end of the analysis.
- Put a standardised solution (titrant) of hydrochloric acid (HCl) or sulfuric acid ($H_2SO_4$) in the burette; this solution will be added (automatically) to the colored boric acid containing the ammonia distilled from the sample. The acid reacts with ammonia in order to measure it.
- Record the volume of the acid titrant solution that was necessary to reach the endpoint and perform a final calculation to find the amount of nitrogen, expressed as % N or % proteins, in the original sample (automatically).

☞ ...with an external potentiometric titrator with a pH electrode (connectable to the UDK 149):

- The titrator burette adds the acid titrant solution automatically to the boric acid solution containing the distilled ammonia, until reaching the endpoint, corresponding to pH = 4.7. In this case we don't check a color change and we don't use indicators, but we follow the corresponding change in the pH of the boric acid solution during the titration process.

☞ ...with a manual colorimetric titration:

- One of the most used indicator solutions is the Tashiro indicator, added to the boric acid solution. The endpoint corresponds to a grey / pink colour. The acid titrant solution is added to the boric acid containing distilled ammonia manually by the analyst, using a glass burette.

☞ ...with a colorimetric method without titration - Nesslerization:

- Often used for low quantities of nitrogen because the spectrophotometric methods are very sensitive. The analyst doesn't perform a titration, but, using the Nessler reagent, obtains a specific colour development whose intensity corresponds to the nitrogen present in the distillate.

**Conclusion**

The Kjeldahl method is extremely versatile, as it can handle a wide range of samples, from Food&Feed (grain, meat, fish, milk, dairy, fruit, vegetables), beverage, environmental (agriculture, oilseeds, soil, fertilizers, water, wastewater, sludge) to chemical and pharmaceutical industries (paper, textiles, rubber, plastic, polymer). In most cases the key to a successful Kjeldahl analysis can be the sample preparation.
This method might not be the fastest method to use but thanks to the high reliability will always give satisfactory results, if performed correctly (following Standards).


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