# Arginase Application Cycle, Conversion Rate, and Carrier Residue Control Strategy

**Application cycle of arginase**

After conversion, filter the arginase and wash it with purified water to remove residual impurities and free protein from the previous batch. Retrieve arginase and continue to use it for the next batch of conversion. We controlled the conversion time to 1.5-8 hours and tested the microbial and bacterial endotoxins, content, other amino acids, and other physicochemical indicators of the finished product of aspartic ornithine produced by arginase (batch number C552306004) continuously applied for 2 months (2023.04.6-2023.06.04). The results showed that the finished product of aspartic ornithine produced by arginase applied for 2 months met the standard requirements. The continuous application of arginase for 2 months can produce finished products that meet quality requirements, so the application period of arginase is temporarily set at 2 months.

| **Test** | **Specification** | C552306001 |
|---|---|---|
| Related substance (TLC) | Spots other than the main spots in the test solution are not larger than the spots obtained in the standard solution | Conform |
| Chloride | ≤ 300 ppm | ＜300ppm |
| Sulfate | ≤ 200 ppm | ＜200ppm |
| Ammonium | ≤ 400 ppm | ＜400ppm |
| Iron | ≤ 30 ppm | ＜30ppm |
| Heavy metals | ≤ 10 ppm | ＜10ppm |
| Sulfated ash | ≤ 0.2 % | 0.03 |
| Assay | 98.0 ~ 102.0 % | 99.2 |
| bacterial endotoxin（EU/mg） | ＜0.006 | ＜0.006 |
| Total plate counts（cfu/g） | ≤ 1000 | 35 |
| Yeast and Mould（cfu/g） | ≤100 | ＜10 |
| E.coli（cfu/g） | Absence | Absence |

**Conversion rate**

According to the experimental report on the application of arginase provided by the arginase manufacturer (Attachment 10-1B), the conversion rate was over 99% after 90

minutes of conversion. The production process controls the conversion time to be above 1.5 hours, and the subsequent crystallization process can further remove the remaining arginine. In addition, other amino acid projects have already been formulated in the finished product, so the conversion rate is not set as a standard for the production process at present.

**Arginase activity:**

The activity of arginase has been confirmed upon entry into the factory. The enzyme activity of arginase for recycling has not been monitored during the current production process. In order to ensure product quality, it is proposed to add a project to investigate the activity of recovered arginase. Inspection frequency: Check once a week during continuous production. The enzyme activity standard is temporarily set at ≥ 2500U/g

**Carrier residue:** Please refer to Attachment 10-1C for the control strategy of carrier residue