Background
Integrity of the mRNA has major impact on the quality of measured expression levels. This is independent of the measurement technique, may it be Next Generation Sequencing (NGS), Quantitative Real-Time PCR (qPCR) or microarray profiling. If mRNA is highly degraded or damaged, measured data will be very unreliable and the whole study is likely a waste of time and money. It is therefore common strategy to test the quality of RNA in samples before conducting large and expensive studies. By using paired qPCR assays that produce amplicons of different lengths from the same target the quality of the RNA can be evaluated. In intact mRNA the Cqs of the short and long amplicons are virtually the same, while for fragmented RNA the Cq of the longer amplicon is increased relative to that of the shorter. The TATAA DeltaAmp Quality Assays consist of short, medium and long qPCR assays that have a common reverse primer for three different targets; Beta-2-Microglobulin (B2M), 18S rRNA (18S) and an Endogenous RNase Resistant marker (ERR). This kit contains three assays for the B2M target.

Instructions for use
The TATAA DeltaAmp Quality Assays are intended to be used to assess the quality of RNA by qPCR measurement and consist of short, medium and long qPCR assays. The difference in Cqs of a longer and shorter assay for the same target reflect the integrity of the RNA in the sample and can be evaluated by the score ΔAmp = CqAmplicon1 – CqAmplicon2 as indicator of RNA integrity. As a second approach amplicon 1 could be the amplicon of an mRNA marker, such as a reference gene (for example the B2M assay), that has normal sensitivity to RNases and amplicon 2 the amplicon of the short assay for the ERR marker. Also in this case the ΔAmp = CqAmplicon1 – CqAmplicon2 can be used as indicator of RNA integrity. For optimal use of these assays it is recommended to compare the ΔAmp-score of the unknown samples to that of sample(s) of known good quality.

For more information, please see:

Storage
The assay can be stored at +4°C for up to 1 month. For long term storage -20°C is recommended. Use within 12 months from arrival. Repeated freeze-thaw cycles should be avoided. Vortex thoroughly and spin down before use.

Content
• Three assays for the B2M target.
  1. B2M short: primer solution for 200 rxn*
  2. B2M medium: primer solution for 200 rxn*
  3. B2M long: primer solution for 200 rxn*

*200 µl of primer mix, C = 10 µM per primer

Protocols, see back.

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**Cycling Protocol**

- UNG step (optional): According to mix instructions
- Enzyme activation: According to mix instructions
- Cycling: According to mix instructions, Annealing temperature: ~60°C

**Pipetting protocol**

<table>
<thead>
<tr>
<th>Component</th>
<th>1 rxn</th>
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</thead>
<tbody>
<tr>
<td>PCR-Grade water</td>
<td>2.6 µl</td>
<td>5.2 µl</td>
<td>6.5 µl</td>
</tr>
<tr>
<td>Primer mix</td>
<td>0.4 µl</td>
<td>0.8 µl</td>
<td>1 µl</td>
</tr>
<tr>
<td>Master mix (2x)</td>
<td>5 µl</td>
<td>10 µl</td>
<td>12.5 µl</td>
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<tr>
<td>cDNA</td>
<td>2 µl</td>
<td>4 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>Final Volume</td>
<td>10 µl</td>
<td>20 µl</td>
<td>25 µl</td>
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