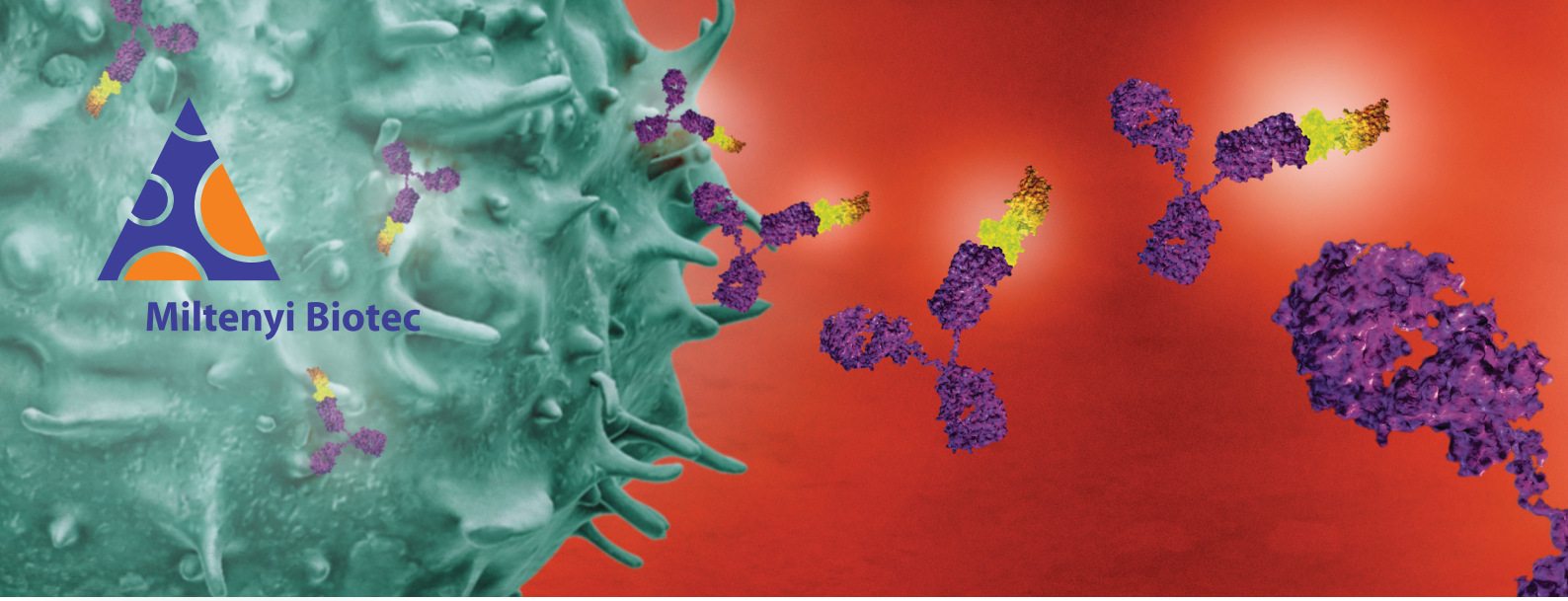



# SARS-CoV-2 research

## Furin cleavage screening assay

### Background

The surface or spike (S) glycoprotein is essential for SARS-CoV-2 attachment and entry into the host cell. While the S1 subunit of the S protein enables binding to the angiotensin-converting enzyme 2 (ACE2) receptor, the S2 subunit mediates membrane fusion. Cleavage at the S1/S2 boundary (amino acid residues R682 to R685) via cellular furin protease primes the S protein for viral entry into the host cell[1]. This furin cleavage site is a novel feature of SARS-CoV-2 in comparison to SARS-CoV-1 and appears to contribute to its higher pathogenicity[2]. Therefore, furin cleavage of the S protein is considered a promising therapeutic target[2,3]. This application note describes how furin cleavage site peptides can be applied in a fluorogenic furin cleavage assay.

### Materials and methods

**Fluorogenic furin cleavage assay**

In the furin cleavage assay, synthetic peptides containing the native SARS-CoV-2 furin cleavage site (SARS-CoV-2 Furin Cleavage Site FAM-Biotin) or a mutated furin cleavage site (SARS-CoV-2 Furin Cleavage Site Control FAM-Biotin) are used to screen for potential furin cleavage–inhibiting molecules (table 1).

The latter control peptide contains amino acid substitutions in the Arg-X-X-Arg furin recognition motif, preventing furin cleavage. All peptides carry an N-terminal FAM (fluorescein amidite) tag, allowing for direct detection via their fluorescent signal without additional labeling. Furthermore, a C-terminal biotin tag enables coupling of the peptides to streptavidin-coated surfaces. Peptides can, for example, be coupled to streptavidin-coated beads and analyzed by flow cytometry, or to streptavidin-coated microplates and analyzed with a plate reader.

First, the optimal concentration of biotinylated peptides to saturate the respective streptavidin-coated surface needs to be determined by titration. Here, PMMA-SAV beads (PolyAn) were used and 10 nM peptide solution was identified as the saturating concentration (data not shown).

To investigate peptide cleavage, the peptide solution (here 10 nM) was incubated with 1 U of furin for 1 h at 25 °C in reaction buffer (as recommended by the manufacturer). Digested peptide solution was then added to PMMA-SAV beads and analyzed by flow cytometry.

Successful furin cleavage could be detected by a shift in FITC fluorescence as depicted in the schematic overview in figure 1. Upon incubation with furin, the N-terminal FAM tag is cleaved off the peptide containing the native cleavage site as opposed to the undigested peptide (without furin) or the control peptide with mutated cleavage site. In addition, inhibition of furin cleavage by furin cleavage–inhibiting molecules, such as EGTA, results in an unaltered FITC fluorescence signal.

| Cleavage site | Peptide sequence and tags | Product | Order no. |
|---|---|---|---|
| Native | FAM-SYQTQTNSP**RRAR**SVASQS-Biotin | SARS-CoV-2 Furin Cleavage Site FAM-Biotin | 130-127-690 |
| Mutated | FAM-SYQTQTNSP**GSAG**SVASQS-Biotin | SARS-CoV-2 Furin Cleavage Site Control FAM-Biotin | 130-127-692 |

**Table 1: Synthetic peptides applied in the fluorogenic furin cleavage assay.** Amino acid sequences and tags of the peptide containing the native furin cleavage site (RRAR) and the control peptide are listed. The control peptide contains a RRAR to GSAG substitution (RRAR682–685GSAG), abrogating the Arg-X-X-Arg furin recognition motif.

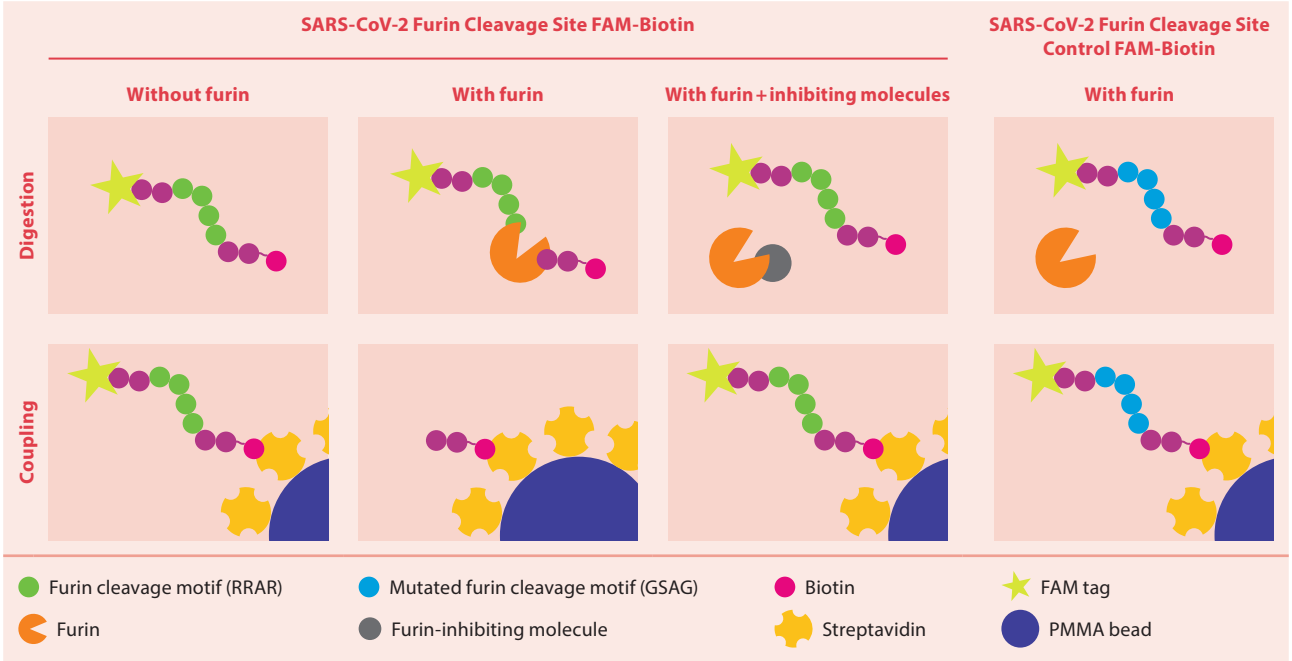



**Figure 1: Schematic overview of the fluorogenic furin cleavage assay.** The native furin cleavage site (RRAR) is illustrated in green and the mutated furin cleavage site (GSAG) of the control peptide in blue. Furin-mediated cleavage results in the removal of the fluorescent FAM tag; whereas the FAM tag remains in place when cleavage is prevented by inhibiting molecules or due to the mutated furin cleavage site (GSAG) of the control peptide. After coupling of the digested peptides to a streptavidin-coated surface via the biotin tag, the fluorescent FAM signal can be analyzed. Only successful cleavage will result in a loss of the fluorescence signal.

## Results

### Successful detection of furin cleavage and furin cleavage inhibition

The peptide with the native furin cleavage site is successfully cleaved, as indicated by the shift in FITC fluorescence intensity after incubation with furin (fig. 2A, B, left). In contrast, the control peptide with the mutated cleavage site cannot be cleaved by furin, resulting in unchanged FITC MFI after incubation with the enzyme (fig. 2A, B, right). SARS-CoV-2 Furin Cleavage Site Control FAM-Biotin can therefore serve as control to screen for potential furin cleavage–inhibiting molecules in the introduced fluorogenic furin cleavage assay.

Moreover, successful detection of cleavage inhibition was shown by adding EGTA, which is known to inhibit the calcium-dependent protease furin. Addition of 10 mM EGTA completely inhibited furin cleavage (fig. 2C), as indicated by unchanged FITC MFI in comparison to the undigested peptide (fig. 2A) and the control peptide (fig. 2, right).

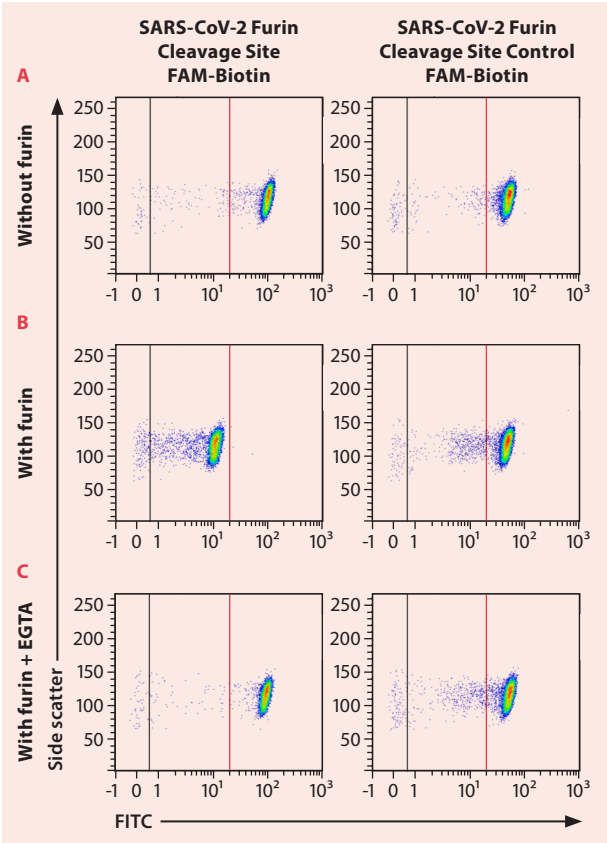



**Figure 2: Successful detection of furin cleavage and inhibition.** Significant reduction of FITC MFI (100→10) shows successful cleavage of the furin cleavage site peptide versus the control peptide (A, B). Unchanged FITC MFI after addition of EGTA demonstrates efficient inhibition of furin cleavage (C).

## Conclusion

- Cleavage of SARS-CoV-2 Furin Cleavage Site FAM-Biotin, containing the native furin cleavage site (RRAR), can successfully be detected by the introduced fluorogenic furin cleavage assay.
- SARS-CoV-2 Furin Cleavage Site Control FAM-Biotin, containing the mutated cleavage site (GSAG), is not cleaved by furin and can thus serve as a control for cleavage site–specific signal reduction.
- The assay can be applied to screen for potential inhibitors of furin cleavage, as shown for inhibition of calcium-dependent furin cleavage by addition of EGTA.

LEARN MORE

Learn more about MACS® Antigens for SARS-CoV-2 research at

▸ **miltenyibiotec.com/SARS-CoV-2-antigens**

## References


1. Coutard, B. *et al.* (2020) The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res. 176: 104742.
2. Johnson, B. A. *et al.* (2020) Furin cleavage site is key to SARS-CoV-2 pathogenesis. bioRxiv. 2020.08.26.268854 Epub ahead of print, Aug. 26.
3. Bestle, D. *et al.* (2020) TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. Life Sci. Alliance 3(9): e202000786.



Miltenyi Biotec

**Miltenyi Biotec B.V. & Co. KG** | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.



Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS and the Miltenyi Biotec logo are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.



V.01